Universidad de Granada
Facultad de Ciencias
Departamento de Zoología

Consejo Superior de Investigaciones Científicas (CSIC)
Estación Experimental del Zaidín (EEZ)
Departamento de Protección Ambiental

Programa de Doctorado en Biología Fundamental y de Sistemas

Tesis Doctoral

Efecto de los hábitats seminaturales del olivar sobre la biología de la familia Chrysopidae (Insecta: Neuroptera)

Rafael Alcalá Herrera
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Memoria que presenta D. Rafael Alcalá Herrera, licenciado en Ciencias Ambientales,

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por la Universidad de Granada

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El doctorando D. Rafael Alcalá Herrera y la directora de la tesis la Dra. Francisca del Carmen Ruano Díaz

Garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por el doctorando bajo la dirección de la directora de la tesis y hasta donde su conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones.

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Contenido

Resumen

Uno de los desafíos de la agricultura del siglo XXI es incrementar su sostenibilidad y reducir su dependencia de los insumos externos. La expansión e intensificación de la agricultura hasta este momento ha provocado una disminución de los hábitats naturales y la biodiversidad, que ha derivado en la pérdida de servicios ecosistémicos de regulación y soporte como el control biológico de plagas. Todo ello ha llevado a la Unión Europea a promover la creación o protección de los hábitats seminaturales (HSNs), para mantener y restaurar la biodiversidad, así como proteger a los organismos beneficiosos importantes asociados. La familia Chrysopidae, con una amplia distribución en casi todos los ecosistemas agrícolas, forma parte del grupo de insectos beneficiosos del cultivo del olivo, donde depredan sobre tres de las dieciocho plagas que inciden en la producción y pueden causar graves pérdidas al olivar, especialmente la polilla del olivo, *Prays oleae* (Bernad, 1788) (Lepidoptera: Yponomeutidae). En este contexto, la conservación e incremento de las poblaciones de crisópidos en el olivar podría mejorar la presión natural sobre las plagas y reducir su dependencia de insumos externos. Los conocimientos adquiridos en esta tesis han permitido abordar aspectos de la biología de la familia Chrysopidae en el estrato arbóreo de los HSNs adyacentes al olivar. Este es un prerrequisito crucial para la elaboración de efectivos programas de manejo del hábitat orientados a la conservación de este valioso depredador.

Tres tipos de metodologías y diseños de muestreo se ejecutaron en cinco olivares ecológicos y sus HSNs adyacentes con el objetivo de establecer el efecto que las especies arbóreas predominantes en estos HSNs tienen sobre la abundancia y diversidad de las especies de la familia Chrysopidae, así como en sus hábitos de oviposición (capítulo 2, 3 y 1). Por ello, el capítulo 1 describe como se localizaron y colectaron mediante observación directa las puestas de crisópidos sobre las copas del olivo y los árboles (almendro, encina y pino) ubicados en los HSNs adyacentes al olivar, para conocer los lugares y formas de oviposición de los crisópidos. La información aportada por el estudio realizado en Agosto de 2016 y 2017, permitió determinar que la comunidad de crisópidos de nuestro biotopo corresponde a especies que depositan sus huevos de forma aislada y en menor medida a especies que lo hacen en racimo. Además se comprobó que el lugar de puesta preferente es el borde y haz de la hoja.

Mediante bandas de cartón y succión (capítulos 2 y 3), se ha determinado la abundancia y diversidad de las fases juveniles y adultos presentes en el olivo, almendro, encina y pino, así como los factores más importantes de reducción de sus poblaciones en las fases juveniles (capítulo 2).

En el capítulo 2 observamos como la tasa de parasitismo fue el factor de reducción de la población de juveniles más importante y que entre las especies de árbol que presentan mayor abundancia de crisópidos (almendro, encina y olivo), el olivo registró la menor tasa de parasitismo. Si a esto le sumamos que en el olivo se contabilizó el mayor número de juveniles que llegaron a alcanzar la fase adulta, el olivo resulta ser la especie de árbol con mayor contribución al número de crisópidos viables en la próxima generación. La comunidad de crisópidos en el biotopo, estudiada a partir de los juveniles eclosionados, la componen diez especies donde *Pseudomallada prasinus* es la especie dominante a lo largo de todo el año, además de ser colectada principalmente sobre el almendro, encina y olivo. En cuanto al complejo parasitario, éste está compuesto por cinco especies, siendo *Baryscapus impeditus* (Eulophidae) la más abundante y parasitando las fases juveniles de *Chrysoperla lucasina*, *Chrysoperla pallida* y *Chrysoperla mutata*, que están asociadas preferentemente al almendro y pino. La segunda especie en importancia fue *Helorus ruficornis* (Heloridae) que parasitó a los juveniles de las especies de *Pseudomallada* y *Cunctochrysa*, las cuales estuvieron asociadas preferentemente a la encina.

En el capítulo 3 demostramos que la encina es la especie más atractiva para la fase adulta de los crisópidos, especialmente para la especie *Pseudomallada prasinus*, que además es la más abundante en el biotopo estudiado. Otras especies importantes de crisópidos por sus abundantes capturas fueron *Chrysoperla mutata* y *Chrysoperla pallida*, que mostraron una preferencia por la encina inicialmente y se fueron desplazando hacia el olivo en otoño. Otra especie destacada fue *Chrysoperla lucasina*, que tiene preferencia por la vegetación herbáceas, y usa el almendro, encina, olivo y pino como lugar de refugio y/o alimentación hasta comienzos del verano (junio, julio y agosto). Aunque no hemos encontrado ninguna asociación entre la abundancia media de larvas y adultos por finca en el olivo con respecto a la depredación media de huevos en la fase antófaga y carpófaga de *Prays oleae* por finca, si observamos una tendencia general del aumento de capturas de larvas sobre el olivo entre junio y julio. Además, no detectamos ninguna asociación entre las cuatro especies de crisópido más abundantes y la dominancia taxonómica de las familias más destacadas y comunes entre las fincas en los dos tipos de HSNs (cubiertas y linde-mancha).

Finalmente, el estudio de cubiertas de herbáceas sembradas con semillas nativas (capítulo 4) fue desarrollado con el objetivo de mejorar nuestro conocimiento sobre la atractividad y el papel funcional de la vegetación herbácea sobre la familia Chrysopidae. Así, 28 de las 42 especies de herbáceas nativas sembradas fueron utilizadas por los crisópidos para refugiarse, reproducirse y/o alimentarse, siendo *C. lucasina* la especie dominante. Entre las presas potenciales disponibles en la cubierta, la abundancia de thrips mostró una correlación positiva con la abundancia de crisópidos. Aunque no observamos preferencia alguna por ninguna especie herbácea concreta en 2016, en 2017, 8 especies registraron abundancias de crisópidos por encima de la media en ese año y cuatro de ellas fueron las más atractivas. Teniendo en cuenta los aspectos entomológicos y agronómicos, las especies más adecuadas para atraer a los crisópidos fueron: *Biscutella auriculata*, *Borago officinalis*, *Silene colorata*, *Crepis capillaris*, *Nigella damascena* y *Papaver rhoeas*.

Introducción general

1.1. Importancia de los hábitats seminaturales en el control biológico por conservación

La intensificación de la agricultura comenzó a principios de 1960 bajo el nombre de "revolución verde" mediante un aumento de la mecanización y el empleo de insumos externos como riego, semillas mejoradas, fitosanitarios y fertilizantes, que ha derivado en el incremento de los servicios productivos (alimentos, forraje, fibras y combustible) y la conversión del paisaje agrícola en extensos monocultivos (Evenson and Gollin 2003). Sin embargo estas prácticas agrícolas han generado una serie de problemas medioambientales de carácter local, regional y global (Matson et al. 1997, Robinson and Sutherland 2002). Entre estos problemas se encuentra la disminución de los hábitats naturales y la biodiversidad (Altieri and Nicholls 2004), que ha conllevado la pérdida de servicios ecosistémicos de regulación y soporte como son el control natural de plagas, la polinización, la fertilidad y la estructura del suelo o el reciclaje de nutrientes (Zhang et al. 2007). Además, existen claras evidencias del efecto perjudicial de los plaguicidas sobre la salud humana (Mostafalou and Abdollahi 2013, Bernieri et al. 2019). Entre los grandes desafíos globales a los que se enfrenta la agricultura en el siglo XXI está el de incrementar su sostenibilidad medioambiental y reducir su dependencia de los insumos externos (McIntyre et al. 2009). Conway (2000) asegura que será necesaria una "segunda revolución verde", en la cual seamos más productivos que en la primera, para alimentar a una población creciente, y más "verde" en la preservación de los recursos naturales y el medio ambiente. Además, los consumidores cada día demandan del sistema agroalimentario alimentos más saludables y seguros (Howard and Allen 2010, GFK 2017).

Todo ello ha llevado a que los estados miembros de la Unión Europea implementen programas medioambientales a través de la Política Agraria Común (PAC), para mantener y restaurar la biodiversidad mediante la creación o protección de hábitats seminaturales (HSNs), que se definen como "cualquier hábitat dentro o fuera del cultivo que contenga una comunidad de plantas no ligadas al cultivo" (Science for Environment Policy 2017). Además, los estados de la Unión Europea en virtud de la directiva (CE) no. 128/2009 están obligados a adoptar los principios de la gestión integrada de plagas, entre los cuales está la "protección y mejora de los organismos beneficiosos importantes" entre los que se encuentran diferentes depredadores y parasitoides, de modo que los HSNs pueden contribuir a su conservación mejorando el control biológico (Holland et al. 2017). La mayoría de los invertebrados en las regiones agrícolas son dependientes de la presencia de HSNs (Duelli and Obrist 2003), porque les proveen de recursos esenciales como néctar, polen, presas alternativas y lugares de refugio, reproducción e hibernación (Holland et al. 2016). Los HSNs deben ser integrados en el paisaje de modo que sean espacial y temporalmente favorables para los enemigos naturales y prácticos de implementar por el agricultor (Landis et al. 2000). Además, los HSNs promueven otros servicios ecosistémicos como la polinización y la conservación del suelo (Wratten et al. 2012).

En el paisaje agrícola europeo existe una amplia variedad de HSNs, que difieren en las especies que lo componen, la estructura vegetal, el área, forma, edad y función, algunos ejemplos son las franjas de arbustos y/o árboles, bordes de bosques y cubiertas herbáceas (Holland et al. 2017). Hay evidencias que muestran como un incremento de la complejidad del paisaje, definido como la cantidad y diversidad o heterogeneidad de hábitats naturales o seminaturales adyacentes al cultivo está asociado con un aumento en la abundancia y diversidad de los enemigos naturales y el control natural de plagas (Thies and Tscharntke 1999, Östman et al. 2001, Bianchi et al. 2006, Chaplin-Kramer et al. 2011, Veres et al. 2013, Rusch et al. 2016, Carpio et al. 2019). No obstante, otros estudios muestran que una alta diversidad de enemigos naturales y paisajes complejos pueden comprometer el control biológico por conservación por el aumento de procesos ecológicos como la depredación intragremial, la redundancia funcional y la complementariedad de nichos (Rosenheim 1998, Martin et al. 2013, Paredes et al. 2013). Además, la importancia de los HSNs puede variar dependiendo del tipo de cultivo, plaga, depredador, el manejo del hábitat y la estructura del paisaje (Tscharntke et al. 2016). Por ello, mejorar el conocimiento de las relaciones entre los recursos disponibles y la composición de artrópodos en los diferentes tipos de HSNs, la distribución espacial y temporal de los recursos en el paisaje y las interacciones con otros factores que juegan un papel en el control de plagas, puede contribuir a optimizar el manejo de los HSNs y el control biológico por conservación (Holland et al. 2016).

1.2. El cultivo del olivo

El olivo (*Olea europaea* L.) empezó a cultivarse en Oriente Medio hace unos 6,000 años y las civilizaciones que poblaron el Mediterráneo lo fueron propagando por ambas orillas (Barranco Navero et al. 2017). En la actualidad la olivicultura se ha expandido a 58 países de los cinco continentes con una extensión total de 11.5 millones de hectáreas (Vilar and Pereira 2018), donde España, y específicamente Andalucía, ocupa el primer puesto con 1,601,295 ha (ESYRCE 2017), convirtiéndose en una excepción geográfica que no se repite en ninguna parte de Europa para una misma especie cultivada (Gúzman-Alvarez et al. 2009).

Antes del siglo XIX era muy frecuente en Andalucía, que el cultivo del olivar estuviera asociado con otros cultivos como naranjos, cerezos, cereal o almendros (Gúzman-Alvarez et al. 2009). Sin embargo, actualmente este tipo de manejo en el olivar solo representa un 3% de su superficie (45,801 ha), destacando el almendro como la opción preferente de asociación con un 81% (37,009 ha), que en la provincia de Granada representa el 58% de la superficie de asociación (4,183 ha) (Junta de Andalucía 2015). Sin embargo, a partir de la segunda mitad del siglo XIX, los olivares andaluces han ido variando las técnicas de cultivo y su mecanización, progresando hacia un monocultivo y disminuyendo progresivamente su asociación con otros cultivos, incrementando el uso del riego cuando es una especie tradicionalmente de secano y volviéndose dependiente del uso de productos químicos para la fertilización y el control de plagas y enfermedades, todo ello con el fin de incrementar las producciones de aceite y aceituna de mesa (Gúzman-Alvarez et al. 2009). Esta simplificación del ecosistema del olivar ha motivado una alteración del medio ambiente con la consecuente pérdida de biodiversidad, suelo e incremento de la contaminación (Ruano et al. 2004, Gómez and Giráldez 2009).

Por otro lado, los consumidores están cada vez más concienciados con la seguridad alimentaria debido a los residuos de pesticidas y muestran una preferencia por el consumo de productos que no los contengan (Eurobarometer 2010, GFK 2017). Además, la Unión Europea a través de su actual PAC, que abarca el período de 2014-2020, tiene como objetivo proteger el medio ambiente y mitigar el cambio climático. Todo ello ha llevado a un fomento de prácticas de cultivo más respetuosas con el medio ambiente, que en el caso del olivar se traduce en tres sistemas de producción certificados y reconocidos por la administración andaluza: producción integrada, agricultura ecológica y producción convencional, que se define por exclusión de los dos anteriores (Garrido et al. 2009).

El sistema de *producción integrada* se define como "aquel que manipula, transforma o elabora productos agrarios utilizando al máximo los recursos y los mecanismos de producción natural, de forma que se garantice una agricultura sostenible. Introduce métodos biológicos y químicos de control, y otras técnicas que compatibilicen las exigencias de la sociedad, la protección del medio ambiente y la productividad agrícola" (Mohedano and Ortiz Berrocal 2011). La Junta de Andalucía fue pionera en implantar esta técnica de producción y el primer reglamento para el olivar se publicó en 1997. Este tipo de producción actualmente se rige por la Orden de 15 de abril de 2008 de la Consejería de Agricultura (Garrido et al. 2009). Mientras que, la *agricultura ecológica* tiene como objetivo producir productos de máxima calidad, conservando la fertilidad del suelo y respetando el medio ambiente, a través de la utilización óptima de los recursos naturales, excluyendo el uso de productos químicos de síntesis (Garrido et al. 2009). Actualmente, a nivel Europeo está regulada por el reglamento (CE) no. 834/2007 y el no. 889/2007. En Andalucía la superficie de olivar dedicada a la agricultura ecológica ha alcanzado las 75,285 hectáreas (PRODESCON 2016).

Entre las dieciocho plagas reconocidas del olivar, debemos destacar tres de ellas como las que más inciden en la producción: *Bactrocera oleae* (Gmelin, 1790), la mosca del olivo (Diptera: Tephritidae), *Prays oleae* (Bernard, 1788), la polilla del olivo (Lepidoptera: Yponomeutidae) y *Saissetia oleae* (Olivier, 1791), la cochinilla del olivo (Homoptera: Coccidae), pudiendo llegar a causar graves pérdidas en el cultivo (Civantos 1995). Los problemas derivados del uso prolongado de insecticidas en el olivar han llevado a la búsqueda de nuevos métodos de control alternativos. Afortunadamente, este cultivo presenta una entomofauna auxiliar muy rica y con gran diversidad de paisajes, que tiene un elevado potencial para la aplicación de técnicas de control biológico por conservación (Paredes and Campos 2014). La familia Chrysopidae desarrolla una actividad depredadora sobre varias plagas relevantes (Porcel et al. 2017), lo que ha generado que sea uno de los depredadores mejor estudiados junto a los sírfidos y los coccinélidos (Duelli 2001).

1.3. Familia Chrysopidae

1.3.1. Posición taxonómica. El complejo Chrysoperla carnea

Actualmente entre los linajes de insectos holometábolos, el superorden Neuropterida es relativamente pequeño en cuanto a diversidad, aunque si tenemos en cuenta su historia evolutiva completa esto no siempre ha sido así (Engel and Winterton 2018). Estudios filogenéticos recientes mediante técnicas moleculares indican que este superorden está compuesto por el orden Raphidioptera, Megaloptera y Neuroptera (Wang et al. 2017, Engel and Winterton 2018).

El orden Neuroptera es el más destacado de los tres en cuanto a riqueza de especies y diversidad morfológica, contiene 5,892 especies agrupadas en 15 familias (Oswald 2018). Dentro del orden Neuroptera, la familia Chrysopidae, es la segunda en cuanto a número y diversidad de especies, compuesta por 1,423 especies válidas recogidas en 82 géneros (Oswald 2018). Actualmente esta familia está dividida en tres subfamilias: Apochrysinae, Nothochrysinae y Chrysopinae. Esta última subfamilia, es la más numerosa en número de especies, alberga el 97% de todas las especies conocidas de crisópidos (Tauber et al. 2009) y está dividida a su vez en cuatro tribus: Ankylopterygini, Belonopterygini, Chrysopini y Leucochrysini (Brooks and Barnard 1990). Recientemente se ha sugerido que la subfamilia Chrysopinae evolucionó como dos linajes monofiléticos, por un lado las tribus Belonopterygini + Leucochrysini y por otro las tribus Ankylopterygini + Chrysopini (Jiang et al. 2017). Además, Haruyama et al. (2008) en su estudio filogenético sobre 24 de los 30 géneros que conforman la tribu Chrysopini (Brooks and Barnard 1990), la divide en siete sub-clados.

Chrysoperla Steinmann, 1964 es uno de los géneros más destacados de la tribu Chrysopini con un elevado interés para su uso en programas de control biológico y donde se han identificado 36 especies mediante características morfológicas con una distribución holártica, que se agruparon en cuatro grupos crípticos de difícil identificación: *carnea*, *comans*, *nyerina* y *pudica* (Brooks 1994).

Hoy en día la situación taxonómica no está totalmente aclarada, ya que algunas especies han quedado bien definidas en base a caracteres morfológicos, hábitats y el canto de apareamiento, pero otras especies son difíciles de asignar sin recurrir a técnicas moleculares. Esto ha llevado a que en el complejo *Chrysoperla carnea sensu lato* se puedan diferenciar 21 especies válidas (Henry et al. 1996, Henry et al. 2002, Canard and Thierry 2007, Henry et al. 2013, Price et al. 2015, Monserrat 2016). Ante esta situación los manuscritos de entomología aplicada han venido optando por referirse a los individuos capturados como *C. carnea s.l.* (Campos and Ramos 1983, Corrales and Campos 2004, Porcel et al. 2017).

Respecto a la situación de la familia Chrysopidae en la Península Ibérica y Baleares se ha publicado una revisión de las 50 especies bien definidas y citadas desde 1972 hasta 2016, donde siete de ellas pertenecen al género *Chrysoperla*; *Chrysoperla agilis* Henry, Brooks, Duelli & Johnson, 2003, *Chrysoperla ankylopteryformis* Monserrat & Díaz-Aranda, 1989, *Chrysoperla carnea sensu stricto* (Stephens, 1836), *Chrysoperla lucasina* (Lacroix, 1912), *Chrysoperla mediterranea* (Hölzel, 1972), *Chrysoperla mutata* (McLachlan, 1898) y *Chrysoperla pallida* Henry et al., 2002, añadiendo además una clave de la fase adulta que permite identificarlos siguiendo caracteres morfológicos y de hábitat (Monserrat 2016).

1.3.2. Ciclo biológico y comportamiento

Las larvas de los crisópidos poseen unas características únicas y fácilmente reconocibles, (i) las mandíbulas y maxilas están fusionadas y forman un canal el cual es empleado para succionar los líquidos de su presa; (ii) el mesodeo y el proctodeo no están conectados, de modo que las heces se acumulan hasta que emerge el adulto del capullo pupal con un sistema digestivo completo y las expulsa en forma de meconio; (iii) los tubos de Malpighi están modificados para la producción de seda por el ano (Withycombe 1925, Gaumont 1976, New 1989).

Actualmente, su distribución biogeográfica es muy cosmopolita, excluyendo a la Antártida, colonizan una gran variedad de ambientes desde tropicales, subtropicales, templados hasta los más extremos como subdesérticos, así como zonas frías y de alta montaña (Greve 1984, New 1986, 1989, Monserrat 2016). De entre todos ellos, el ecosistema agrícola es el más estudiado debido a la gran importancia económica que tienen en el control biológico, donde las tres familias más destacadas son Chrysopidae, Hemerobiidae y Coniopterygidae (Canard et al. 1984, Stelzl and Devetak 1999, McEwen et al. 2001)

Entre los crisópidos existen dos tipos de larvas: desnudas (por ejemplo el género *Chrysoperla*) o cubiertas con restos de elementos externos procedentes de los restos de sus presas, su propia exuvia o restos vegetales (por ejemplo el género *Pseudomallada* Tsukaguchi, 1995, *Rexa* Navás, 1920 y *Cunctochrysa* Hölzel, 1970). Esta capa les permite defenderse de las hormigas, camuflarse para acercase a sus presas potenciales y podría ser usada como barrera física frente a los parasitoides (Smith 1921, 1926, Kennett 1948, New 1969, Eisner and Eisner 2002, Monserrat and Diaz-Aranda 2012). Además otro comportamiento defensivo de la larva es segregar por el ano sustancias paralizantes, para evitar la depredación, o sustancias tóxicas disuasorias almacenadas tras la ingesta de sus presas (Kennett 1948, Blum et al. 1973, Rothschild et al. 1973, Monserrat 1980, New 1986, Güsten and Dettner 1991, Monserrat 2015).

Las larvas son depredadores polífagos, que se alimentan de una amplia variedad de pequeños insectos de cuerpo blando y ácaros, así como huevos y pequeñas larvas de lepidópteros (Ridgway and Murphy 1984). La presa es localizada por contacto y reconocida mediante receptores táctiles en el extremo de las maxilas y receptores químicos en el extremo final de los palpos labiales y las antenas (Canard and Volkovich 2001). En el caso de *C. carnea s.l.* los estímulos visuales y olfativos podrían ayudarles a encontrar las presas (Ables et al. 1978, Sengonca et al. 1995). Después del contacto físico y su reconocimiento, la larva captura la presa con una serie de movimientos característicos, insertando una de las mandíbulas en ella para succionar sus líquidos internos y con la otra mandíbula la manipula (Canard and Duelli 1984).

La fase larvaria pasa por tres estadios con dos cambios de muda y tras haber alcanzado su completo desarrollo en el tercer estadio larvario, al final de su etapa de depredación activa, deja de ingerir alimento durante uno o dos días para iniciar la fase de pupación, en la cual mediante la seda producida por una secreción elaborada en los tubos de Malpighi y exudada por el ano, fabrica un capullo con forma esférica o semiesférica, tardando en esta labor entre uno y dos días (Smith 1922, Principi 1940, Díaz-Aranda 1992, Canard and Volkovich 2001). En la superficie del capullo se pueden encontrar las setas de la larva y para aquellas especies que portaban restos de elementos externos, también los incorpora quedando el capullo total o parcialmente cubierto (Monserrat and Diaz-Aranda 2012). Los lugares seleccionados para la fabricación del capullo pupal son variados y cada especie tiene sus preferencias, algunas de ellas lo hacen en la planta hospedadora y otras lo hacen en el suelo (Canard 2001, Monserrat and Diaz-Aranda 2012). Por ejemplo *Pseudomallada flavifrons* (Brauer, 1851) o *Pseudomallada prasinus* (Burmeister, 1839) prefieren lugares abiertos como el tronco, las ramas o la superficie de las hojas (Lacroix 1924, Principi 1956) y *Chrysopa pallens* (Rambur, 1838) prefiere las hojas curvadas e infestadas de áfidos (Principi 1940). Una vez tejido el capullo pupal, la larva adopta una posición quiescente y curvada en fase de prepupa, tras lo cual puede mudar inmediatamente y permanecer en estado de pupa, o bien mudar a pupa unos días antes de emerger del capullo. En cualquier caso la pupa es exarata y déctica, la muda de prepupa a pupa es detectada porque la exuvia de la larva de tercera generación es desplazada al polo caudal y se observa un disco de color pardo (Canard and Principi 1984, Díaz-Aranda 1992).

El periodo más crítico en el ciclo biológico del crisópido es el transcurrido entre la emergencia de la pupa madura del capullo pupal y el adulto, la pupa madura emerge del capullo a través de un corte transversal en el polo cefálico opuesto al polo caudal, y tras emerger busca un soporte vertical donde llevar a cabo la muda de la cutícula pupal a adulto (Smith 1922, Principi 1940, Diaz-Aranda and Monserrat 1992, Canard and Volkovich 2001).

Monserrat (2016) describe una serie de características distintivas de los adultos como su cabeza hipognata con manchas sobre las estructuras cefálicas, antenas moniliformes generalmente más cortas que las alas anteriores y aparato bucal masticador. En el tórax es habitual observar una banda media dorsal más pálida que a veces se extiende sobre el abdomen. Las alas son membranosas y transparentes con venación abundante y compleja, oscilan entre 6-35 mm de longitud y en estado de reposo adoptan la típica posición en tejadillo o incluso se sitúan paralelas al sustrato para camuflarse, el abdomen compuesto por diez segmentos está poco esclerificado y contiene abundante setación.

Los adultos emergidos como norma general pasan por una primera fase de pre-emparejamiento, en la cual sus gónadas maduran en un tiempo variable según la especie, el sexo y los factores climáticos, y buscan pareja o se emparejan para copular. Luego tenemos una segunda fase de pre-ovoposición donde la hembra inseminada deposita los primeros huevos, ambas fases en la bibliografía suelen confundirse y referirse únicamente como fase de “pre-ovoposición” (Canard and Volkovich 2001). Los adultos una vez emergidos del capullo en esta fase de su ciclo realizan un vuelo de migración durante 2-3 noches, son vuelos largos en la dirección del viento e independientemente de las fuentes de alimentación o las posibilidades de emparejamiento (Duelli 1980b, a). Después de este período, los adultos realizan vuelos tanto a favor como en contra del viento al verse atraídos por los estímulos químicos asociados a las fuentes de alimento y para emparejarse, esta actividad se produce después del atardecer, debido a su actividad crepuscular hasta poco antes de amanecer, con picos de actividad entre las 22-24 h (Duelli 1980b, a).

Se ha demostrado que existe una cierta asociación entre el tipo de planta elegida como sustrato y las especies de crisópidos (Monserrat and Marín 1994, 2001). La mayoría de los crisópidos adultos de la familia Chrysopidae siguen una dieta glicófaga procedente del néctar y melazas de plantas e insectos (Devetak and Klokocovnik 2016), algunos géneros combinan esta dieta con polen, son los llamados palino-glicófagos (*Chrysoperla*, *Pseudomallada*) (Villenave et al. 2005, Villenave et al. 2006) o bien son palinófagos estrictos (*Hypochrysa* Hagen, 1866, *Kimochrysa* Tjeder, 1966, *Pamochrysa* Tjeder, 1966 y *Pimachrysa* Adams, 1956) (Tjeder 1966). Por último algunos crisópidos presentan una dieta omnívora (*Anomalochrysa* McLachlan, 1883, *Atlantochrysa* Hölzel, 1970, *Chrysopa* Leach, 1815) formada por ácaros, pequeños insectos fitófagos, polen, levaduras y esporas de hongos (Canard 2001).

La familia Chrysopidae se caracteriza por depositar sus huevos sobre el sustrato en el extremo de un largo y fino pedúnculo de naturaleza proteica (excepto los del género *Anomalochrysa* endémico de Hawái), con una longitud de hasta 15 mm (Diaz-Aranda and Monserrat 1992). Los huevos son ovales de entre 0.70-2.73 mm de longitud para los géneros europeos y su coloración generalmente es verdosa o blanquecina (Monserrat 2016). La función del pedúnculo es incierta, se contemplan varias posibilidades como la protección ante el canibalismo, los parasitoides (Smith 1922, Duelli 1984, 1986, Duelli and Johnson 1992), los depredadores (Chen and Young 1941) y los impactos y/o humedad (Lacroix 1921). Generalmente las hembras tienden a realizar la ovoposición en aquellos lugares cercanos a potenciales fuentes de alimento para su descendencia, facilitando la localización de presas por parte de las larvas neonatas, aunque esta conexión es menos acentuada para *C. carnea s.l.* (Duelli 1984). Las formas de las puestas pueden ser aisladas, en grupos o en racimo, esto depende de la especie y los lugares más frecuentes son el haz, envés y borde de las hojas, aunque también se han encontrado en el tronco de los árboles, un caso particular son las coníferas donde las puestas suelen encontrarse en el extremo apical de las acículas (Pantaleoni 1996, Szentkirályi 2001a, Monserrat 2016).

En nuestras latitudes, la mayoría de las especies de crisópidos de los géneros *Chrysoperla*, *Chrysopa* y *Pseudomallada* parecen tener ciclos bi-trivoltinos, desarrollándose durante los meses de primavera y verano, incluso ciertas especies de algunos géneros (*Chrysoperla* y *Pseudomallada*) muestran un solapamiento de las generaciones mostrando actividad a lo largo de todo el año. Por el contrario, algunas especies de varios géneros son claramente monovoltinas (*Rexa*, *Nineta* Navás, 1912, *Peyerimhoffina* Lacroix, 1920, *Nothochrysa* McLachlan, 1868, *Italochrysa* Principi, 1946 e *Hypochrysa*), aunque especies polivoltinas pueden convertirse en monovoltinas en función de las condiciones climáticas locales, por ejemplo *Chrysopa* o *Chrysoperla* (Canard 1987, Canard and Vannier 1992, Volkovich 1998, Szentkirályi 2001a, Monserrat 2016). Todo este desarrollo generacional se ve controlado a su vez por la diapausa, que en el caso de la familia Chrysopidae se produce sobre la fase larvaria y el adulto (Principi 1991).

1.3.3. La familia Chrysopidae y el olivar

La comunidad de crisópidos en el agroecosistema del olivar ha sido estudiada en numerosos países, donde el método de muestreo preferente son las trampas McPhail, aunque también hay estudios donde se hace uso de otros métodos de captura como cazamariposas, insecticidas, trampas de pegamento, vareo, succión, etc. (Canard et al. 1979, Alrouechdi et al. 1980b, Alrouechdi et al. 1980a, Neuenschwander and Michelakis 1980, Neuenschwander et al. 1981, Campos and Ramos 1983, Alrouechdi 1984, Porcel et al. 2017). Una revisión de los estudios realizados en el olivar por Szentkirályi (2001b) contabilizó 32 especies de crisópidos diferentes; 13 en la península ibérica, 16 en Francia, 12 en Italia y 18 en Grecia, en donde las especies dominantes fueron *C. carnea* s.l. y *P. flavifrons*. Otras especies frecuentes en casi todos los estudios aunque menos abundantes fueron: *P. prasinus*, *Pseudomallada zelleri* (Schneider, 1851) y *Pseudomallada genei* (Rambur, 1842). Entre el resto de especies habría que destacar a *Rexa almerai* (Navás 1919), que muestra una preferencia exclusiva por los olivares de la península ibérica y de Francia (Campos and Ramos 1983, Monserrat and Marín 1994). Desde la perspectiva del control biológico sería importante tener en cuenta aquellas especies de crisópidos que utilicen el olivo para reproducirse y no solo como refugio o para alimentarse, prestando atención tanto a los estadios preimaginales como al adulto. En este sentido se podrían destacar las especies del complejo *C. carnea s.l.*, y del género *Pseudomallada* (*P. prasinus* y *P. flavifrons*).

En relación con su actividad depredadora en el olivar varios estudios han puesto de manifiesto que los crisópidos son los mayores depredadores oófagos de *P. oleae*, revelando una relación entre la abundancia de adultos, larvas y puestas de crisópidos con la presencia de larvas en la generación antófaga y huevos en la generación carpófaga de esta plaga (Neuenschwander and Michelakis 1980, Alrouechdi et al. 1981, Campos 1989). Además, ensayos serológicos sobre larvas de *C. carnea s.l.* colectadas en dichos períodos han demostrado esa relación depredador-presa (Morris et al. 1999). También ensayos de comportamiento de las tres fases larvarias de *C. carnea s.l.* confirmaron una respuesta positiva frente a la depredación de huevos de *P. oleae* (Alrouechdi 1981). De las tres generaciones que posee *P. oleae*, la generación carpófaga es la que presenta valores más elevados de huevos depredados, donde en algunas zonas, el porcentaje de frutos con toda las puestas depredadas alcanza una media del 57% (Ramos and Ramos 1990).

Así pues, los crisópidos son considerados importantes agentes de control biológico en el olivar, por lo que diferentes estudios van dirigidos a conservar e incrementar sus poblaciones, así como su eficacia depredadora (Campos 2001). En uno de estos estudios, se consiguió atraer adultos de crisópidos mediante el uso de melazas artificiales, sin embargo, esto no se tradujo en un incremento de sus poblaciones en el cultivo debido a la falta de sustancias proteicas necesarias para la producción de huevos y tampoco se observó un incremento sobre la depredación de huevos de *P. oleae* (Liber and Niccoli 1988, McEwen et al. 1994). Por otro lado, se ha determinado que los HSNs del olivar juegan un papel importante en la ecología de los crisópidos ya que usan la cubierta vegetal para reproducirse (McEwen and Ruiz 1994) y que ésta tiene un efecto positivo en su diversidad y abundancia, coincidiendo con la presencia de *P. oleae* en el cultivo y sugiriendo la posibilidad de que los crisópidos podrían moverse hacia el cultivo en el momento en el que se produzca un ataque de la plaga (Porcel et al. 2017). Otros estudios han determinado los recursos, relacionados con los hábitats naturales asociados al olivar, que estos enemigos naturales pueden utilizar para alimentarse, así como la incidencia sobre sus características biológicas (longevidad y reproducción) (Gonzalez et al. 2016, Nave et al. 2016, Villa et al. 2016).

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Objetivos

Los objetivos de la presente tesis doctoral son:

Objetivo 1. Profundizar en diversos aspectos de la ecología de la familia Chrysopidae sobre el cultivo del olivar y el estrato arbóreo de los hábitats seminaturales (HSNs) adyacentes (almendro, encina y pino):

> **Objetivo 1.1.** Investigar si existe algún patrón específico en la ovoposición de los adultos en relación a las cuatro especies arbóreas estudiadas.
>
> **Objetivo 1.2.** Evaluar los factores de reducción de las poblaciones de juveniles de crisópidos en los distintos árboles estudiados.
>
> **Objetivo 1.3.** Determinar la atractividad del cultivo y el estrato arbóreo de los HSNs sobre la abundancia y diversidad de adultos de la familia Chrysopidae y su relación con la abundancia de plagas (*Prays oleae* (Bernard, 1788)) y la presencia de determinadas especies de plantas.

Objetivo 2. Analizar la atractividad de ciertas especies herbáceas nativas sobre la familia Chrysopidae y su potencial uso en la restauración de cubiertas en los agroecosistemas perennes Mediterráneos.

Material y Métodos general

3.1. Área de estudio

Los estudios se realizaron en cinco olivares ecológicos y su hábitat seminatural (HSN) adyacente (capítulos 1, 2 y 3) ubicados en los términos municipales de Deifontes y Píñar (Figura 1, Tabla 1), pertenecientes a la comarca de los Montes Orientales. Esta comarca está localizada en el extremo norte de la provincia de Granada, a unos 20 km de la capital, limítrofe con la provincia de Jaén y con las comarcas de la provincia de Granada: Guadix, Vega, Sierra Elvira y Alfanevada. Además, un estudio sobre cubiertas herbáceas sembradas (capítulo 4) se llevó a cabo en una finca experimental, en la pedanía de Villarrubia del municipio de Córdoba, situada en la comarca de la Vega del Guadalquivir (Figura 1).

La topografía de los Montes Orientales presenta en un 32% de su superficie (395 ha), pendientes inferiores al 10% y en el 24% (296 ha), las pendientes superan el 25% (Junta de Andalucía 2014). El 68% del suelo (839 ha), de los 14 municipios que conforman la comarca, está dedicado a la agricultura, siendo la superficie media de las explotaciones agrarias de 15.2 ha y el cultivo del olivar el predominante. Además el 31% del suelo (382 ha) son hábitats seminaturales (HSNs) (Junta de Andalucía 2014). El bioclima de la comarca es pluviestacional-oceánico, con un termotipo mediterráneo superior, un ombrotipo subhúmedo inferior y un térmico templado fresco. Su temperatura media anual es de 14^{o}C, con marcadas fluctuaciones de temperatura entre unos inviernos muy fríos con mínimas bajo cero, y unos veranos muy cálidos con temperaturas por encima de 29^{o}C. La precipitación media anual es de 672.3 mm, que se concentra entre octubre y mayo, por el contrario los meses estivales, entre junio y agosto, suelen ser marcadamente secos (Valle Tendero et al. 2005, Junta de Andalucía 2018). El suelo está formado por asociaciones de cambisoles cálcicos con regosoles calcáreos, litosoles y rendsinas (IUSS Working Group 2015).

Los olivares ecológicos se encuentran a una altitud similar entre 800 y 1,100 metros sobre el nivel del mar (m s. n. m.), con un rango de pendientes del 11 al 16% y sus superficies van desde 0.9 a 215 ha (Tabla 1). La variedad de aceituna es picual y el marco de plantación (8 x 8 y 12 x 12 metros) es muy parecido. La incidencia de plagas como la polilla del olivo, *Prays oleae* (Bernard, 1788), y la mosca del olivo, *Bactrocera oleae* (Gmelin, 1790), y enfermedades como el repilo, *Fusicladium oleagineum* (Cast.) Hughes, se solventó con la aplicación puntual y localizada de productos recogidos en el Anexo II del reglamento (CE) no. 889/2008. Todos los olivares mantienen una cubierta espontánea, que fue eliminada mediante siega mecánica y/o pastoreo entre los meses de abril y mayo. Además en el período de postcosecha se abonó con materias de origen orgánico y se incorporaron los restos de poda triturados en las calles del cultivo, creando una cubierta inerte.

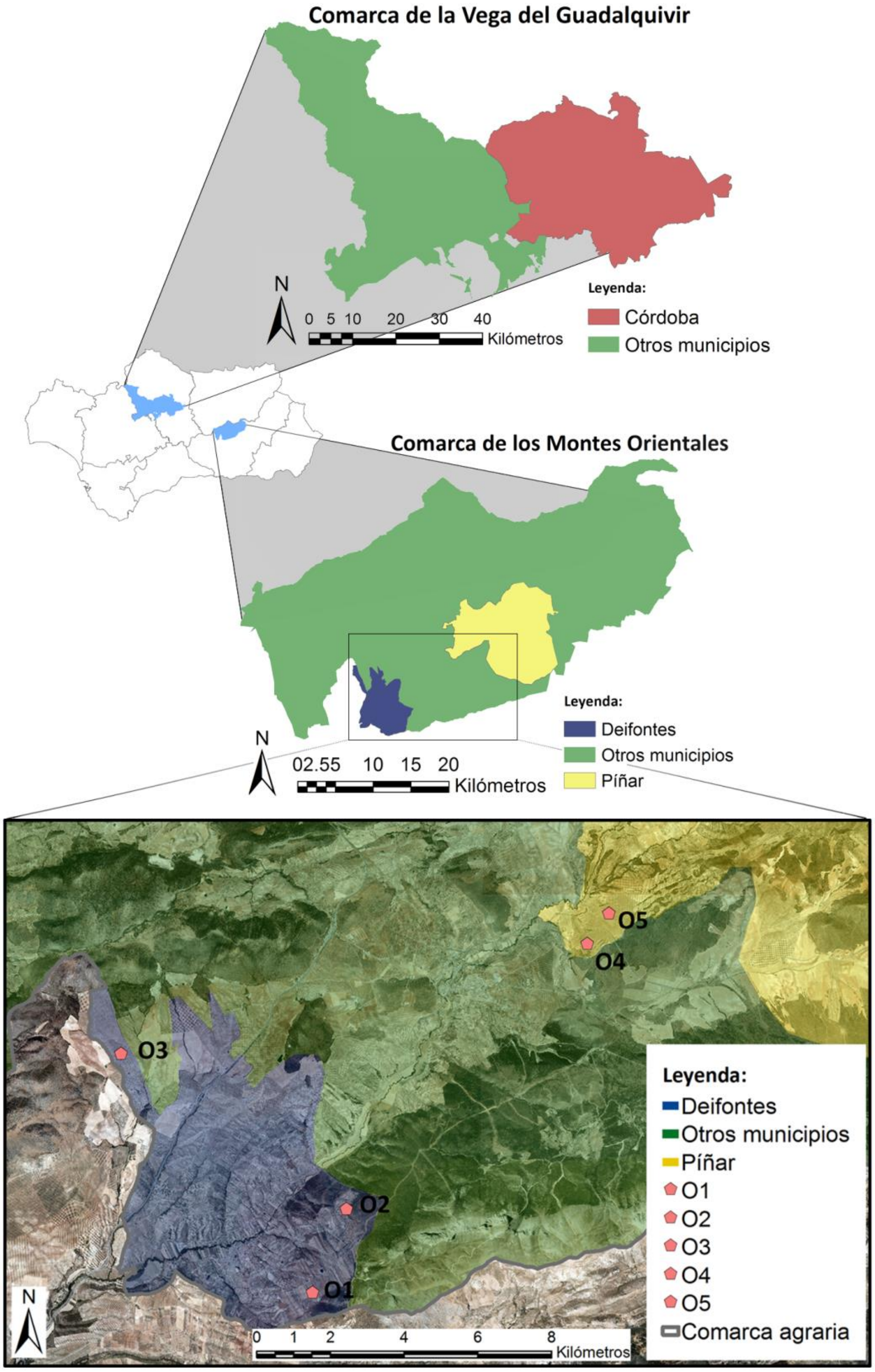

Figura 1. Ubicación de las comarcas, municipios del área de estudio y los olivares ecológicos (O1 a O5) seleccionados de la comarca de los Montes Orientales.

Tabla 1. Características de los olivares ecológicos seleccionados en la comarca de los Montes Orientales.

	Olivar 1 (O1)	**Olivar 2 (O2)**	**Olivar 3 (O3)**	**Olivar 4 (O4)**	**Olivar 5 (O5)**
Denominación	Norberto	La Pedriza	Los Almendros	Píñar derecha	Píñar izquierda
Ubicación	Deifontes	Deifontes	Deifontes	Píñar	Píñar
Coordenadas	37°19'5.96"N; 3°34'9.92"O	37°20'17.44"N; 3°33'39.21"O	37°22'24.76"N; 3°37'46.03"O	37°24'14.29"N; 3°29'14.13"O	37°24'40.93"N; 3°28'52.41"O
Altitud (m)	1,010	960	840	880	880
Superficie (ha)	4.3	0.9	215	58	124
Pendiente (%)	11	12	15	16	11
Marco de plantación (m)	8x8	8x8	8x8	12x12	12x12
Aplicación de fitosanitarios	Cobre	Cobre	Cobre, *Bacillus thuringensis*	Cobre, *Bacillus thuringensis*, Spinosad	Cobre, *Bacillus thuringensis*, Spinosad
Fertilización	Abono orgánico Restos de poda	Abono orgánico, Restos de poda	Compost, Restos de poda	Abono orgánico, Restos de poda	Abono orgánico, Restos de poda
Riego	Goteo	Goteo	Secano	Goteo	Goteo
Tipo de HSNs	Cubierta, linde y mancha de vegetación	Cubierta, linde y mancha de vegetación	Cubierta, linde y mancha de vegetación	Cubierta, linde y mancha de vegetación	Cubierta, linde y mancha de vegetación
Manejo cubierta	Siega mecánica	Siega mecánica	Siega mecánica	Pastoreo y siega mecánica	Pastoreo y siega mecánica

Olivar 1 (O1): En la cubierta de este olivar predominan las especies *Crepis vesicaria* L., *Rostraria cristata* (L.) Tzvelev, y *Erodium primulaceum* (Lange) Lange. Además las lindes están compuestas principalmente por *Prunus dulcis* (Mill.) D. A. Webb y *Quercus rotundifolia* Lam., y al fondo del olivar existe una mancha de vegetación donde destacan las especies *Pinus halepensis* Mill., *Cistus albidus* L. y *Macrochloa tenacissima* (L.) Kunth (Figura 2 y 3).

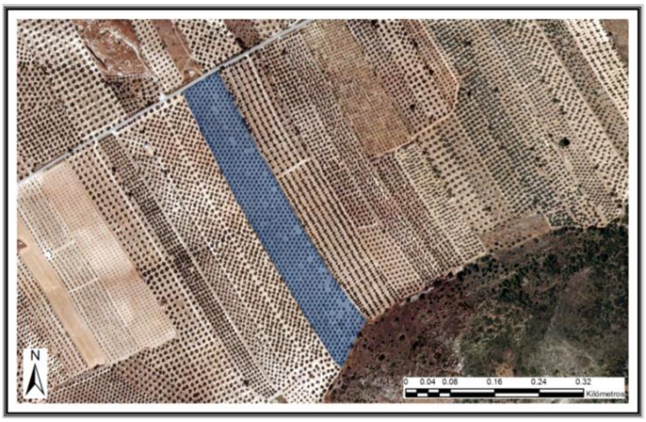

Figura 2. Ortofoto del olivar denominado Norberto sombreada en azul.

Figura 3. Vista del olivar denominado Norberto.

Olivar 2 (O2): En este olivar las especies reseñables de la cubierta son: *Erodium malacoides* (L.) L´Hér. subsp. *malacoides*, *Geranium molle* L. y *Trifolium tomentosum* L.. El olivar limita al sur con una mancha de vegetación donde destacan las especies *P. halepensis*, *M. tenacissima*, *Brachypodium retusum* (Pers.) P. Beauv. subsp. *retusum* y *Q. rotundifolia* (Figura 4 y 5).

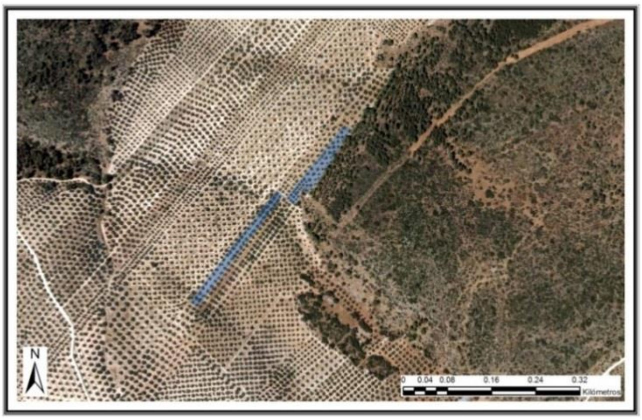

Figura 4. Ortofoto del olivar denominado La Pedriza sombreada en azul.

Figura 5. Vista del olivar denominado La Pedriza.

Olivar 3 (O3): Su cubierta está formada esencialmente por *C. vesicaria*, *Plantago lanceolata* L., *Anacyclus clavatus* (Desf.) Pers., y *Diplotaxis erucoides* (L.) DC.. En los bordes de los caminos hay individuos de *P. dulcis*. Por el oeste el olivar limita con un arroyo que contiene especies típicas de ribera. Por último en la parte superior existen dos manchas de vegetación y al principio del olivar hay otra, compuestas fundamentalmente por *Q. rotundifolia*, *Genista cinerea* (Vill.) DC., *B. retusum* subsp. *retusum* y *Rhamnus lycioides* L. (Figura 6 y 7).

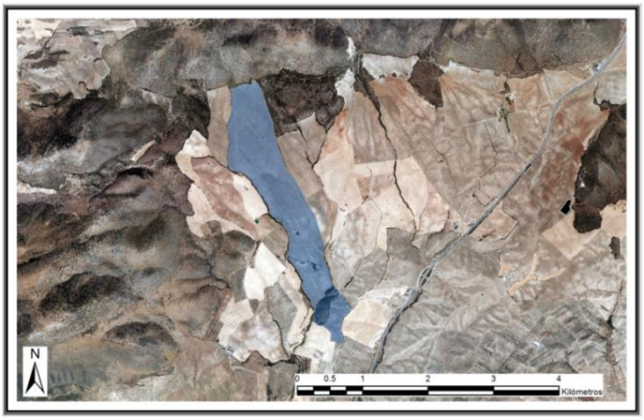

Figura 6. Ortofoto del olivar denominado Los Almendros sombreada en azul.

Figura 7. Vista del olivar denominado Los Almendros.

Olivar 4 (O4): Una mezcolanza de *Leontodon longirrostris* (Finch & P. D. Sell) Talavera, *Medicago minima* (L.) L., *Medicago rigidula* (L.) All. y *G. molle*, componen la cubierta de este olivar. Además el olivar contiene manchas de vegetación en el interior, una mancha adyacente que la limita por el sur (que conecta con la mancha de vegetación de los olivares O1 y O2) y una linde por el oeste, donde resaltan las especies *P. halepensis* (está al interior de la mancha que limita por el sur con el olivar, en las coordenadas 37°21'20.77"N; 3°32'04.59"O), *Q. rotundifolia*, *Quercus coccifera* L., *M. tenacissima* y *Rosmarinus officinalis* L. (Figura 8 y 9).

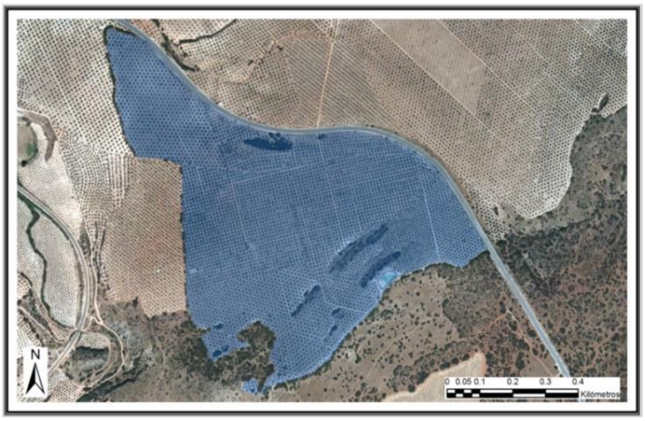

Figura 8. Ortofoto del olivar denominado Píñar derecha sombreada en azul.

Figura 9. Vista del olivar denominado Píñar derecha.

Olivar 5 (O5): La cubierta está integrada esencialmente por *M. minima* y *L. longirrostris*. Otras infraestructuras ecológicas que contiene son dos manchas de vegetación al norte y al este, además tiene una linde arbórea al oeste y al norte, entre todas ellas las especies más destacadas son: *M. tenacissima*, *Q. rotundifolia*, *R. officinalis* y *Thymus zygis* L. subsp. *gracilis* (Boiss.) R. Morales (Figura 10 y 11). Además en la zona norte hay plantadas dos líneas de *P. dulcis*.

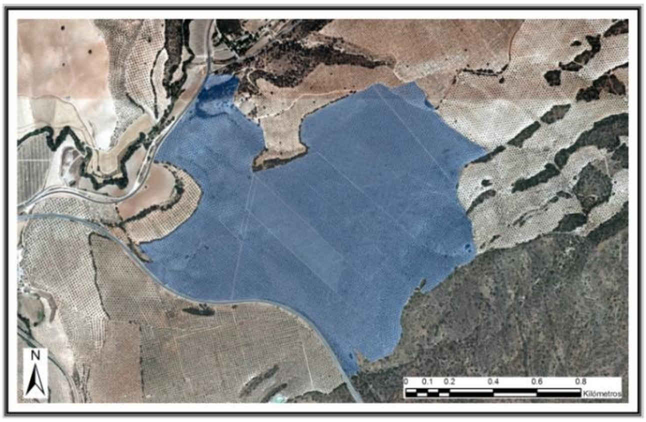

Figura 10. Ortofoto del olivar denominado Píñar izquierda sombreada en azul.

Figura 11. Vista del olivar denominado Píñar izquierda.

Finca experimental: Esta finca conocida como "Cortijo El Naranjal" se encuentra a una altitud de 86 m s. n. m. y sus coordenadas son 37°49'47.83"N, 4°54'21.47"O. Posee una extensión de ocho hectáreas y una topografía prácticamente plana (1%). Está rodeada, al norte, este y oeste, por cultivos de naranjos y tierras arables, y al sur por vegetación de ribera y el río Guadalquivir.

3.2. Técnicas y diseño del muestreo

En el transcurso de esta tesis se han usado tres técnicas de muestreo para la captura de especímenes de la familia Chrysopidae, en la fase preimaginal (huevo, larva, prepupa-pupa) y en la fase adulta (capítulo 1, 2, 3 y 4). Además, se monitoreó la presencia e incidencia de *P. oleae* en sus tres generaciones y en la fase adulta (capítulo 3). A continuación se aportan datos generales sobre las técnicas de muestreo, para mayor detalle le remitimos al correspondiente capítulo de la tesis.

Observación directa: Esta técnica permitió colectar las puestas de crisópidos en las copas de *P. halepensis* (pino), *P. dulcis* (almendro), *Q. rotundifolia* (encina) y *Olea europaea* L. (olivo) durante 2016 y 2017 (capítulo 1). Esta técnica ya había sido utilizada por Campos (1989) y Alrouechdi (1980) sobre olivo. Un total de 45-50 árboles de cada especie (Figura 12) seleccionados aleatoriamente (con una separación mínima entre árboles de 15 metros para asegurar la independencia espacial) fueron muestreados según su disponibilidad en el área de estudio. Las copas de los árboles se inspeccionaron, hasta una altura de 160 a 170 centímetros, durante un minuto, colectando los órganos vegetativos (hojas, ramillas, frutos y/o botones florales) en donde se observaron puestas de crisópidos.

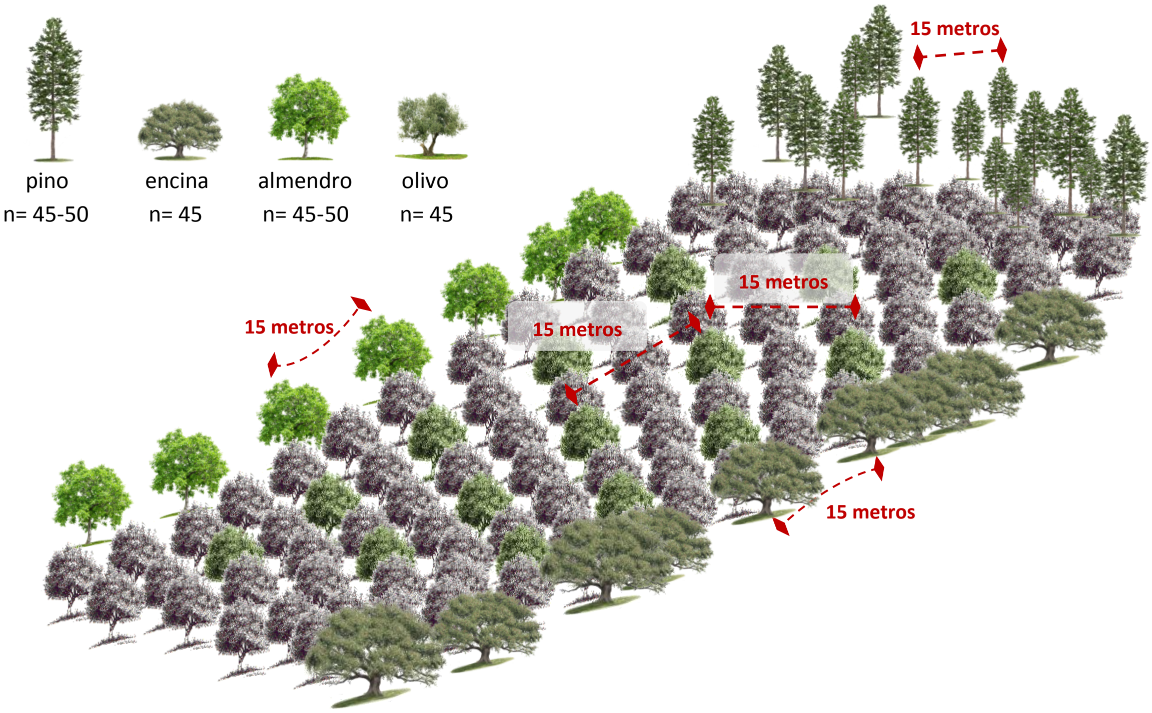

Figura 12. Diagrama del diseño muestral mediante observación directa en 2016 y 2017.

Bandas de cartón: Este método fue empleado para colectar las fases juveniles (larva, prepupa-pupa) de los crisópidos durante 2016 y 2017, ya que tienden a refugiarse en estas bandas de cartón. Estudios previos hicieron uso de las bandas de cartón para conocer la diversidad de crisópidos sobre el olivo, manzano, naranjo y nogal (Clancy 1946, Alrouechdi 1980, Campos 1989). El capítulo 2 describe como se instalaron ocho trampas de cartón corrugado sobre 25 árboles por muestreo de almendro, encina, olivo y pino (con una distancia mínima entre árboles de 15 metros para asegurar la independencia espacial) y cuya distribución dependió de su disponibilidad en el área de estudio (Figura 13). Las bandas de cartón se situaron en ramas diferentes a 160 y 170 centímetros sobre el suelo, repartidas en los cuatro puntos cardinales (dos bandas de cartón por orientación).

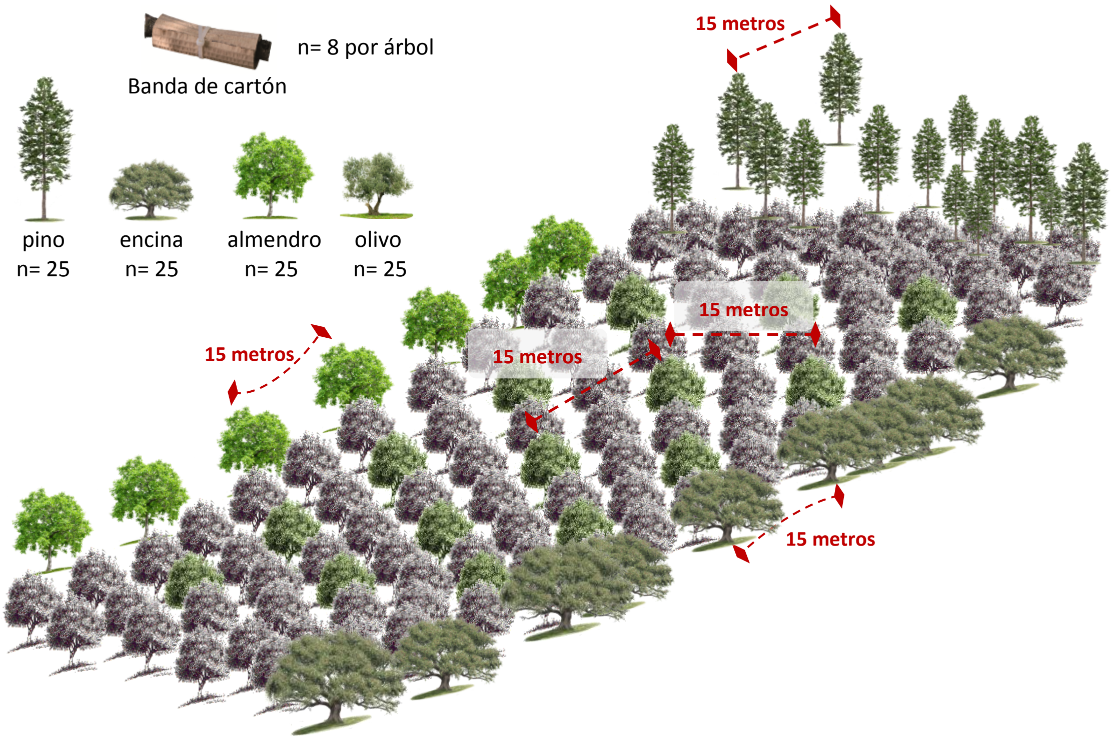

Figura 13. Diagrama del diseño muestral mediante bandas de cartón corrugado en 2016 y 2017.

Succión: La captura de crisópidos (larva y adulto) en 2016 se realizó mediante aspiradora entomológica (InsectaZooka; BioQuip®, Rancho Dominguez, CA, USA). En estudios anteriores se ha probado la efectividad de la aspiradora entomológica para colectar crisópidos y pequeños artrópodos (Wilson et al. 1993, Macleod et al. 1994, Villenave et al. 2005, Paredes et al. 2013, Sorribas et al. 2016, Porcel et al. 2017, Rodríguez et al. 2017, Cotes et al. 2018). En la succión (capítulo 3) se aspiró la copa de 75 árboles por muestreo de almendro, encina, olivo y pino, aleatoriamente seleccionados (con una distancia mínima entre árboles de 15 metros para asegurar la independencia de las muestras) y distribuidos de forma equitativa según su disponibilidad entre los cinco olivares y sus HSNs (Figura 14). La copa era aspirada rodeando por completo el árbol, hasta una altura de tres metros durante dos minutos, para realizar un muestreo homogéneo. En la boca de la aspiradora se colocaba un calcetín de poliamida que actuaba como malla de interceptación de la artropofauna, una vez pasado el tiempo de succión los calcetines eran etiquetados e inmediatamente guardados en una nevera con hielo para prevenir la interacción entre los artrópodos capturados.

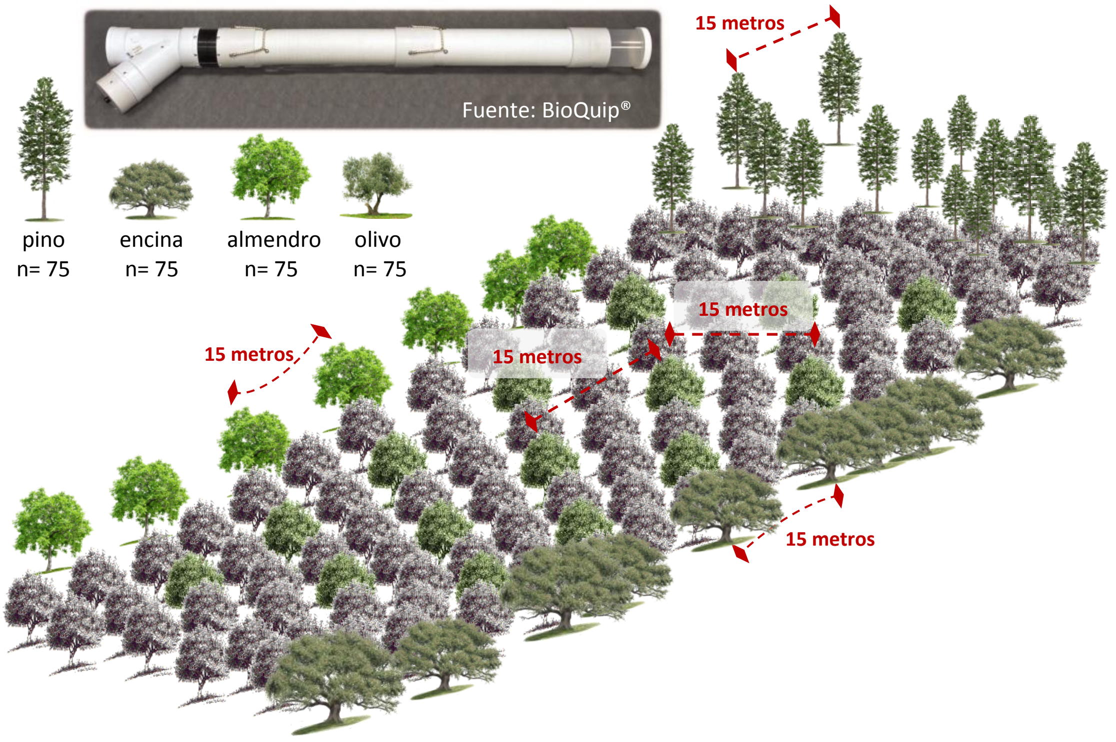

Figura 14. Diagrama del diseño muestral mediante succión para el estrato arbóreo en 2016 y de la aspiradora

En el capítulo 4 se vuelve a emplear la misma técnica de muestro pero esta vez sobre cubiertas herbáceas sembradas. En 2016 se sembraron aleatoriamente, un total de 40 especies en parcelas de nueve metros cuadrados (3x3 m) separadas entre sí por 1.75

metros, en tres bloques con una separación entre bloques de cinco metros (Figura 15). Cada parcela se aspiró durante 40 segundos en el período óptimo de floración. Mientras que en 2017 (Figura 16) se sembraron aleatoriamente 22 especies en una única parcela, con un rango de superficie entre 107 y 5,640 m^2, sin separación entre ellas. En cada parcela se tomaron tres muestras (al principio, mitad y final de cada parcela) de 40 segundos. Una vez pasado el tiempo de succión los calcetines eran etiquetados e inmediatamente guardados en una nevera con hielo para prevenir la interacción entre los artrópodos capturados.

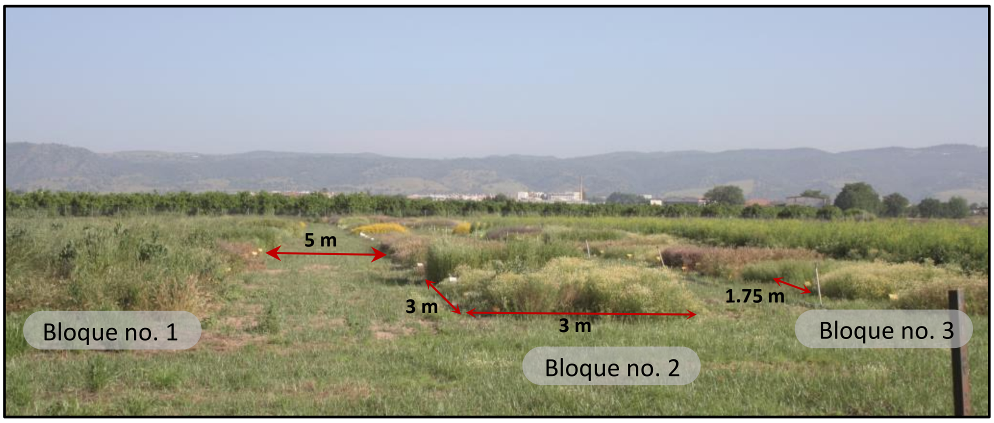

Figura 15. Vista del diagrama muestral de las muestras de succión en las cubiertas herbáceas en 2016

Figura 16. Vista del diagrama muestral de succión en las cubiertas herbáceas en 2017

Seguimiento e incidencia de P. oleae: Durante 2016 la fase adulta de *P. oleae* se monitoreó en los cinco olivares mediante la instalación de dos trampas funnel por olivar (Econex polillero TA027; Sanidad Agrícola Econex S.L., Santomera, Murcia, España) con atrayente sexual (Snailnex®; Sanidad Agrícola Econex S.L., Santomera, Murcia, España) y una separación mínima entre cada trampa de 150 metros (Figura 17). El contenido de cada trampa era guardado en campo en un frasco roscado de 150 ml. Además el estudio del daño provocado por la generación filófaga, antófaga y carpófaga de *P. oleae* (Figura 18) se realizó mediante la recolección de diez brotes al azar de un palmo de longitud, sobre cinco a diez olivos aleatoriamente seleccionados, dejando un árbol sin muestrear entre dos árboles muestreados para asegurar la independencia espacial de las muestras tomadas.

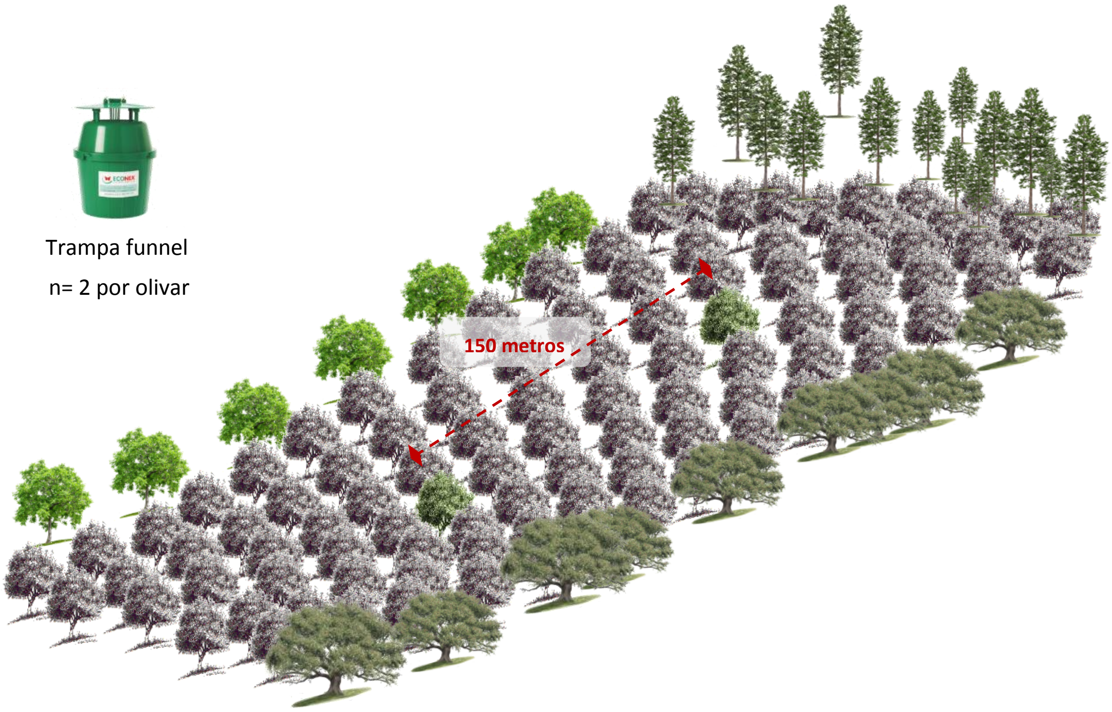

Figura 17. Diagrama del diseño muestral de adultos con trampas funnel en 2016.

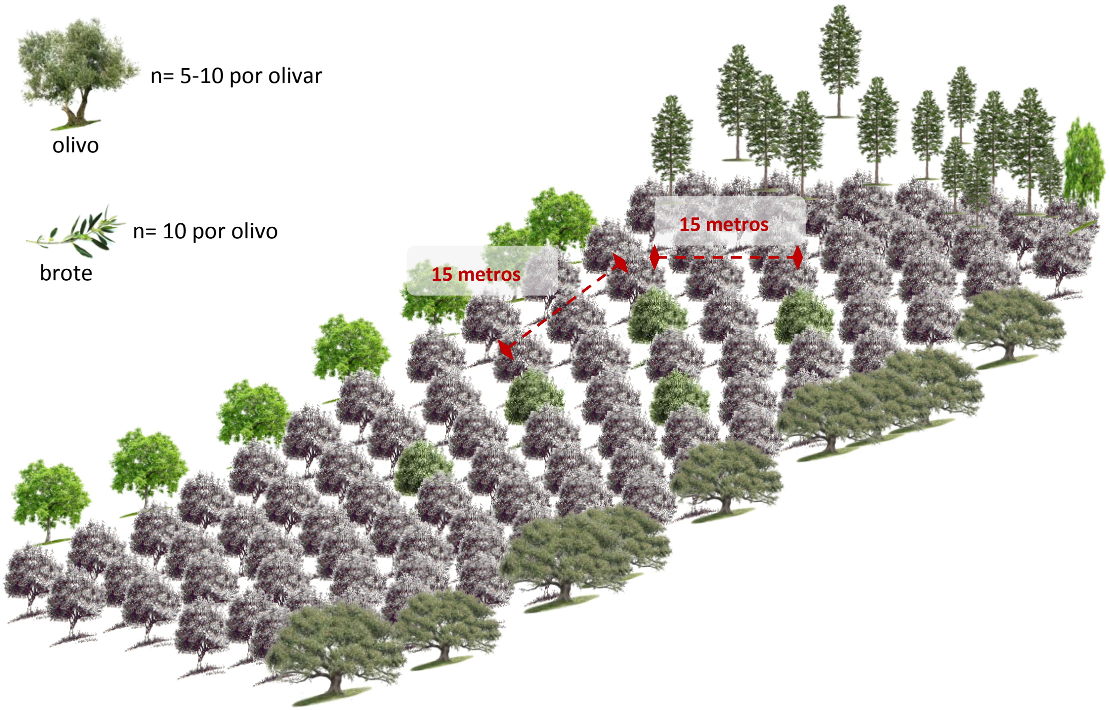

Figura 18. Diagrama del diseño muestral de las generaciones de *P. oleae* en 2016.

Flora y vegetación: El muestreo de las cubiertas en los olivares se efectuó mediante el método no destructivo de número de contactos "*Point Quadrat*" (Daget and Poissonet 1971) (Figura 19.A), en un transecto lineal de cinco metros con un punto de contacto cada cinco centímetros (100 puntos de contactos por transecto), en 2016. En cada olivar se realizaron cuatro transectos en la calle y otros cuatro bajo la copa del olivo. Mientras que para los otros HSNs (linde-mancha de vegetación), dado que sufren menos perturbaciones y el porte de las especies vegetales es mayor a las que nos encontramos en la cubierta, se utilizó el método "*Line Intercept*" (Canfield 1941) (Figura 19.B), en 2017. Este método consistió en anotar el rango de interceptación de la especie vegetal (en centímetros) con una línea de 25 metros trazada hacia el interior de la mancha o a lo largo de la linde. El número de transectos por linde-mancha era de tres con una distancia mínima de 50 metros entre cada uno, en cambio el número total de linde-mancha entre los cinco olivares seleccionados varió entre dos y seis. Los especímenes vegetales de dudosa identificación *in situ* en ambas metodologías (*Point Quadrat* y *Line Intercept*) fueron etiquetados para su posterior identificación en el laboratorio.

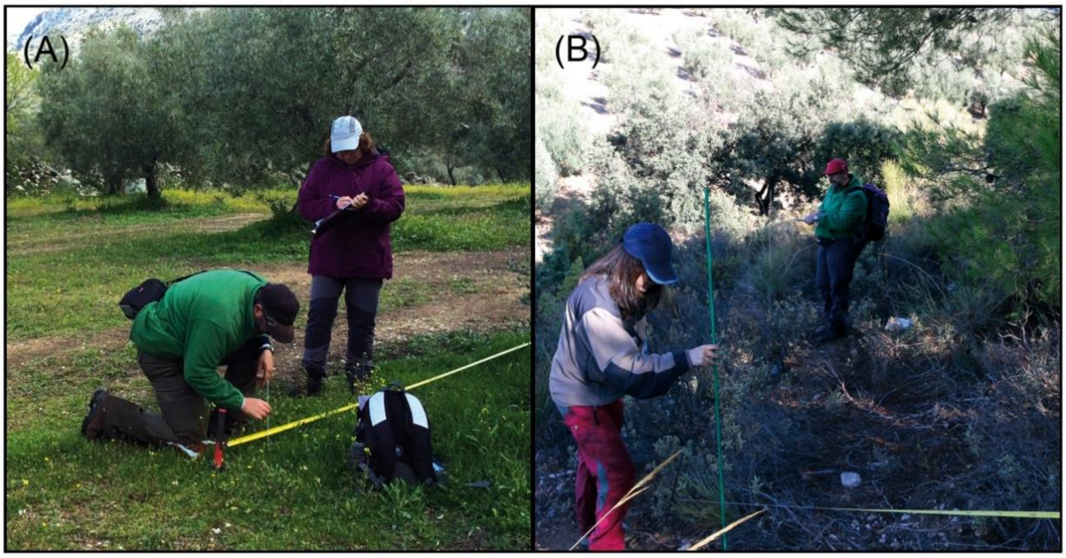

Figura 19. Muestreo de flora y vegetación mediante el método "*Point Quadrat*" en la cubierta (A) y el método "*Line Intercept*" en la linde-mancha de vegetación (B).

3.3. Procesado de las muestras de campo en el laboratorio

Observación directa: Las puestas de crisópidos recogidas mediante observación directa en 2016 fueron individualizadas en placas de Petri (55 mm de diámetro), etiquetadas e incubadas en cámara (Fitoclima s600 PLH; Aralab, Rio Mouro, Portugal) a una temperatura de 25±1°C, una humedad entre 50-60% y un fotoperiodo de 16:8 (D:N) horas. A continuación las larvas emergidas de las puestas se alimentaron *ad libitum* con huevos de

Ephestia kuehniella Zeller (Lepidoptera: Pyralidae) (EphestiaTop; Biotop, Livron-sur-Drôme, Francia) hasta comenzar la fase de pupación con el fin de que completaran su ciclo biológico hasta adulto y poder ser identificadas.

Bandas de cartón: Las fases juveniles (larva, prepupa-pupa) colectadas de cada banda de cartón eran individualizas en placas de Petri (55 mm de diámetro) etiquetadas e incubadas en cámara (Fitoclima s600 PLH; Aralab, Rio Mouro, Portugal) a una temperatura de 25±1°C, una humedad del 50-60% y un fotoperiodo de 16:8 (D:N) horas, para monitorear su evolución. Las larvas se alimentaron *ad libitum* con huevos de *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (EphestiaTop; Biotop, Livron-sur-Drôme, Francia) hasta comenzar la fase de pupación para que pudieran completar su ciclo biológico hasta adulto y poder ser identificadas taxonómicamente. El adulto de crisópido emergido del capullo pupal se conservó a -20°C hasta su identificación, mientras que los parasitoides emergidos del capullo pupal se etiquetaron y conservaron en tubos de centrífuga de 0.6 ml con alcohol al 70% hasta su identificación.

Succión: Las muestreas de campo eran almacenadas a -20°C hasta el momento de su limpieza. El contenido de las muestras se vaciaba en bandejas de poliestireno y bajo estereomicroscopio (Nikon SMZ 800; Nikon, Tokyo, Japón) se separaban cuidadosamente los crisópidos del resto de artropofauna y el material vegetal. Los crisópidos eran individualizados, etiquetados, guardados en tubos de centrífuga de dos ml y conservados a -20°C hasta su identificación. El resto de artropofauna se etiquetó y conservó en tubos de centrífuga de dos o cinco ml con alcohol al 70%.

Todos los adultos obtenidos mediante los distintos métodos de muestreo (observación directa, bandas de cartón y succión), se identificaron taxonómicamente bajo estereomicroscopio hasta el nivel de especie siguiendo las claves contenidas en Monserrat (2016), además ciertos individuos le fueron consultados al autor de las claves para aclarar los caracteres morfológicos que permitieran su identificación. En cambio, los parasitoides se identificaron hasta especie con la ayuda de expertos taxónomos (ver agradecimientos), la comprobación con la colección de referencia del Grupo de Protección Vegetal de la Estación Experimental del Zaidín y la clave de Goulet and Huber (1993).

Seguimiento e incidencia de P. oleae: El contenido de los botes roscados de las trampas funnel se vaciaba en bandejas de poliestireno y bajo estereomicroscopio se separaba cuidadosamente todo aquel artrópodo que no fuera la fase adulta de *P. oleae*. Una vez se disponía del contenido limpio se cuantificó el número de adultos capturados. Por otra parte los brotes de olivo recolectados en cada generación de *P. oleae* se conservaron a 4^{o}C para evitar su deshidratación y se inspeccionaron bajo estereomicroscopio. En la generación filófaga se contabilizó el daño causado en las hojas y la edad de las larvas de *P. oleae*. En la generación antófaga, se seleccionaron aleatoriamente dos inflorescencias por brote y se contabilizaron los huevos depositados según su estado (incubación, eclosionados y depredados). Mientras que en la generación carpófaga se repetía la acción pero en dos frutos por brote.

Flora y vegetación: Los especímenes dudosos de los muestreos de campo eran prensados en el laboratorio e identificados hasta nivel de especie por el Dr. Antonio García mediante las claves de la Flora Vascular de Andalucía Oriental (2011).

Todos los datos generados en esta tesis fueron almacenados en dos bases de datos creadas exclusivamente para este proyecto, una para los datos entomológicos y otra para los datos de flora y vegetación.

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Late summer oviposition of green lacewings (Neuroptera: Chrysopidae) on olive groves and adjacent trees

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4.1. Abstract

In this study we assess how chrysopids (generalist predators widely used as biological control agents) use olive, almond, oak and pine trees for oviposition. We sampled clutches of chrysopids from tree canopies in August of 2016 and 2017. Additionally, we checked the stage (alive, predated, emerged or parasitized) and the site (upper side, edge or underside of leaves) and mode (single, and cluster clutches) for oviposition in every clutch. The clutches collected alive in August 2016 were raised in the laboratory and identified to species level. No differences in clutch abundance were observed amongst tree species, years and locations, suggesting that chrysopids are geographically well spread and that all of the tree species are equally suitable to support clutches of active chrysopids in this period. Additionally, chrysopids preferred to lay their clutches on the edge and upper side of the leaves and the single clutches were the most frequent. We collected clutches of nine chrysopid species belonging to four genera, from which *Pseudomallada prasinus* (Burmeister, 1839), *Cunctochrysa baetica* (Hölzel, 1972) and *Chrysoperla mutata* (McLachlan, 1898) were the most abundant. The almond trees shelter the highest chrysopid species richness. We found *C. mutata* as the only *Chrysoperla* Steinmann, 1964 species laying on the olive trees and *Chrysoperla lucasina* (Lacroix, 1912) and *Chrysoperla pallida* Henry et al., 2002 on the almond trees. Although every tree species is able to shelter a similar abundance of eggs of chrysopids, conserving different trees ensures the existence of a diverse assemblage of chrysopids that might respond to periodical perturbations and moves to olive groves in the case of a pest outbreak.

Keywords: natural vegetation; ecological infrastructure; biological control by conservation.

4.2. Introduction

Chrysopidae is an important family of the Neuroptera Order because of their widespread habitat distribution and their role as predators in ecosystems (Szentkirályi 2001a, c) especially the larvae (McEwen et al. 2001, Nicoli Aldini 2002, Pappas et al. 2011). Adults are fully winged with crepuscular or nocturnal activity (Duelli 1986a). The majority of adults have a palyno-glycophagous diet composed of vegetable products (nectar, pollen) and even insect honeydew (Canard et al. 1984, Canard 2001, Villenave et al. 2005). The eggs (clutches) are laid as single (one egg), group or cluster clutches (5-40 grouped eggs with separated pedicels or forming a bouquet) and can often be found on the upper side, underside, tips or edges of the leaves of different plants (Duelli 1984b, Monserrat 2016).

Moreover, Chrysopidae is one of the Neuropteran families with a high number of species, c. 1,200 species grouped in 86 genera/subgenera (Aspöck et al. 1980, Brooks and Barnard 1990, Winterton and Brooks 2002) some of them very difficult to identify from their morphological traits, but possible from their courtship songs and genetics (Brooks 1994, Henry et al. 1999, Lourenço et al. 2006). Thus, one of the most cited chrysopids, *Chrysoperla carnea,* has been reported as a complex of species, of which at least seven are present in our study area. They are difficult to identify but they have some morphological differences recently reviewed by Monserrat (2016) for the Iberian species. A similar situation occurs with other genera such as *Pseudomallada* Tsukaguchi, 1995 or *Cunctochrysa* Hölzel, 1970 (Monserrat 2008, Canard and Thierry 2017, Duelli et al. 2017). Some of these species pertaining to the same complex could be differentiated by their type of oviposition as occurs between *Pseudomallada prasinus* (single eggs) and *Pseudomallada marianus* (cluster clutches) (Monserrat 2016).

Lacewings are differently attracted to vegetation; some of them prefer more vertical plants (bushes or trees) and other species prefer herbaceous plants (Nielsen 1977, Aspöck et al. 1980, Sziraki 1996, Monserrat and Marín 2001, Duelli et al. 2002). It has been previously established that some of the adult chrysopid species, such as *Chrysoperla carnea s.l.* (Stephens, 1836), have been collected from a broad range of plant species (Monserrat and Marín 1994, 2001). However, other chrysopid species show a clear preference for a determined substrate, for instance: *Chrysoperla mediterranea* (Hölzel, 1982) to pines or *Pseudomallada prasinus* (Burmeister, 1839) to oaks (Monserrat and Marín 1994, 2001). There could be several reasons for this attraction of the adults, such as the volatiles produced by each plant and/or its associated pests, the existence of potential prey for the brood (Duelli 1984b, Szentkirályi 2001a) or even the architecture of the flowers and the accessibility of resources in the plant for the chrysopid adults (Nave et al. 2016), amongst others.

The olive is a native crop of the Mediterranean region, currently cultivated around the world in zones with a Mediterranean climate, where it is one of the most important cultivated fruit crops. *C. carnea s.l.* has been considered the dominant neuropteran species in this crop (Alrouechdi et al. 1980, Campos and Ramos 1983, Pantaleoni and Curto 1990, Porcel et al. 2017), showing a significant predation effect on populations of the three most important olive pests: *Prays oleae* (Bernard, 1788), *Saissetia oleae* (Olivier, 1791), and *Euphyllura olivina* (Costa, 1839) (Campos 2001) together with a relatively high ability to develop resistance to insecticides (Jansen 2000).

In the past, olive groves were frequently mixed with other crops such as almonds, especially in fields without irrigation in south-eastern Andalusia. Nevertheless, currently, most of the farms are eliminating the noncrop trees to facilitate all the olive management labor (Gúzman-Alvarez et al. 2009). Very limited and accurate data about the presence of chrysopids in almond trees is available, with only data about the abundance of the family Chrysopidae and the presence of *C. carnea s.l*. as the most abundant species (Hoy et al. 1979, Almatni and Khalil 2008, Yanik et al. 2011, Sánchez-Ramos et al. 2017).

Pine and oak trees are common in patches of natural vegetation and the edges surrounding olive groves in Spain. Marín (1994) found four species of chrysopids in pine habitats in Spain, in which *C. carnea* and *C. mediterranea* were the most abundant species. The chrysopid fauna of oak forests in Spain has also been studied showing a high richness of species (eight) and *P. prasinus* and *C. carnea* were the most abundant (Marín and Monserrat 1987, Marín 1994, Monserrat and Marín 1994).

Late-summer (August) has been signaled in several studies as a period of high abundance of adult chrysopids (Marín and Monserrat 1987, Marín 1994). In fact, in a long-term study on chrysopids, Campos (1989) found that the period of June to September presented the highest density, and so it could be considered a good period to sample this insect group.

Currently, landscape management is one of the techniques used in the programs of biological control by conservation. The goal of such programs is to increase the natural enemy populations in the adjacent vegetation to the crops (Boccaccio and Petacchi 2009, Paredes et al. 2013). The proximity of natural enemies ensures their movement to the crops when a pest outbreak occurs. Chrysopids have been previously detected in the surroundings of olive groves by baited food and sticky traps in oak and pine forests close to olive groves (González et al. 2008).

Thus, it is essential to investigate the ecology of the Chrysopidae family in depth and their relationship with the landscape around olive groves to get a precise idea about what and how olive and adjacent trees are used for reproduction, in order to protect the population of this insect family and its predatory role in the olive groves. For instance, the oviposition site and mode can have an impact on chrysopid fitness, because of a different exposition to climate and management factors. Moreover, these oviposition traits could help to identify different species pertaining to the same complex. Therefore, we have tried to find out which are the active summer chrysopid species, how oviposition occurs in olive groves and in the more abundant adjacent trees during late summer and also to identify the different species laying on each of these trees.

4.3. Materials and Methods

We have sampled 45-50 randomly selected trees for the four species: *Olea europaea* L. (olive), *Prunus dulcis* (Mill.) D.A. Webb (almond)*, Pinus halepensis* Mill. (pine)*,* and *Quercus rotundifolia* L. (oak), from April to August 2016 we visited the same sampling locations and inspected a total of 185 trees searching for clutches, in the Montes Orientales area (Granada, Andalusia, Spain) (Table 1). The canopy of each tree was observed at a height of 160 to 180 cm for one minute (direct observation) to collect the chrysopid clutches. The total observed clutches per month between April to July were insufficient (4 and 55) to give conclusive results on all the trees. We only detected an increased number of clutches on the leaves of all the trees in August 2016, and then we repeated the sampling in August 2017.

The landscape in the Montes Orientales area is similar to all Andalusia, dominated by olive groves (1.6 million ha of olive groves in Andalusia, ESYRCE 2017) with only some edges and few seminatural areas in the surrounding containing oak, almond and/or pine trees. The best distributed tree species, olive and oak trees, were sampled in five different locations whilst pine and almond trees were not homogenously distributed in the study area and were sampled in three different locations depending on the tree availability. Thus, we sampled a total of five different organic farms including their corresponding edges and seminatural areas (Norberto 37°19'5.96"N; 3°34'9.92"W; La Pedriza 37°20'17.44"N;

3°33'39.21"W; Los Almendros 37°22'24.76"N; 3°37'46.03"W; Píñar 37°24'40.93"N; 3°28'52.41"W; Punto intermedio 37°21'20.77"N; 3°32'04.59"W). These farms were into an area of 8 Km of radius and agrochemicals were used following the organic policies (CE 889/2008 and CE 834/2007). Selecting organic farms enabled us to ensure the most natural conditions for the chrysopid populations included in our study.

The whole collected clutches from the August 2016 campaign were transported to the laboratory and checked under a stereoscopic zoom microscope (Nikon SMZ 800; Nikon, Tokyo, Japan) to determine the type of clutches (single, group or cluster) and the position: on the leaf (upper side, underside, edge) and on other locations (over leave pedicels, twigs, and flower buds), which were very scarce and then grouped under the term “others” (other locations). We also took into account the egg stage: alive (without any opening and a yellow-green color), predated (empty egg and with an irregularly broken cover), parasitized (brownish color or/and with a regular circular opening) or chrysopid emerged (empty egg with a transversal opening) following Johnson and Bin (1982).

Table 1. Number of samples collected per tree species, location and year.

			2016				**Total**
	Location 1	n	Location 2	n	Location 3	n	n
Almond	Los Almendros	12	Los Almendros	8	Norberto	25	45
Oak	Los Almendros	15	Píñar	15	Norberto	15	45
Olive	Los Almendros	15	Píñar	15	Norberto	15	45
Pine	Punto intermedio	13	La Pedriza	12	Norberto	25	50
			2017				**Total**
	Location 1	n	Location 2	n	Location 3	n	n
Almond	Los Almendros	15	Los Almendros	10	Norberto	25	50
Oak	Los Almendros	15	Píñar	15	Norberto	15	45
Olive	Los Almendros	15	Píñar	15	Norberto	15	45
Pine	Punto intermedio	10	La Pedriza	10	Norberto	25	45

The clutches collected in 2016 were individually stocked in Petri dishes (55 mm. diameter) and maintained in an incubation chamber (Fitoclima S600 PLH; Aralab, Rio de Mouro, Portugal) at 25±1^{o}C, 50-60% RH and a photoperiod of 16:8 h (L:D). After hatching, the larvae were separated individually, reared and fed on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (EphestiaTop; Biotop; Livron-sur-Drôme; France) (Porcel et al. 2011). All the emerged adult lacewings were pinned for later identification under stereomicroscope to species level following Monserrat (2016).

4.3.1. Statistical analysis

Count data on the abundance of the clutches per tree species, location, year, oviposition site, and mode were expressed as the average number of clutches per tree.

Data analysis started with a data exploration, following the protocol described by Zuur et al. (2010). The average number of clutches was fitted to a generalized linear model (GLM) (Formula 1) with a negative binomial distribution (η_i) as a function of the covariates: year, location, tree species, oviposition site and mode, and an offset of the number of sampled trees in each location (Zuur et al. 2013). The interactions amongst the tree species with the oviposition site and mode were not included in the model because we did not have data for these variables in all the tree species. Collinearity was not found between the variables used in the model and their variance inflation factors (VIF) were less than three. The predictor function was:

$$\eta_i = \text{Intercept} + \text{year}_i + \text{location}_i + \text{trees species}_i + \text{oviposition site}_i + \text{oviposition mode}_i + \text{offset} \qquad (1)$$

The backwards selection procedure was used as model selection and the Akaike Information Criterion (AIC) was used as an effective technique for selecting the factors influencing the abundance of clutches (Burnham and Anderson 2002).

The significant variables in the model were compared in each tree species by Kruskal Wallis tests. When significant differences were found, a post hoc Nemenyi test was applied to make comparisons between multiple factors.

All the statistical analyses were performed with R software version 3.4.3 (R Development Core Team 2017). “PMCMR” package (Pohlert 2014) was used to calculate the pairwise multiple comparisons in Kruskal-Wallis test, and “multcomp” package (Hothorn et al. 2008) was used to calculate the pairwise multiple comparisons in GLM (Tukey test).

4.4. Results

In total, 796 clutches were recorded by direct observation and collection in August 2016 (455) and 2017 (341). The mean abundance of clutches found per tree was not different between both study periods (0.6±0.09 and 0.56±0.13 clutches/tree, respectively), among locations, and among tree species (Table 2). Nevertheless, we found a significantly different mean abundance of clutches per tree when taking into account the oviposition site and mode (Table 2).

Table 2. Comparison of the mean abundance of clutches per tree for the different covariates by ANOVA (GLM). Significance of variables in the model is indicated as follows: n.s - non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

	Degree of freedom variable	Degree of freedom model	Residuals deviance	*p* value (>|Chi|)	
Null		87	205.835		
Year	1	86	205.515	0.57144	n.s
Location	6	80	197.466	0.23450	n.s
Tree species	3	77	190.023	0.05904	n.s
Oviposition site	3	74	99.931	<2.2e-16	*p*<0.001
Oviposition mode	1	73	80.032	8.165e-06	*p*<0.001

4.4.1. Oviposition sites

In general, the mean number of clutches was significantly high on the edge and the upper side of the leaves (1.04±0.16 and 0.66±0.14, respectively, without significant differences between both), compared with the mean of clutches laid on the underside of the leaves (0.21±0.05) and in “others” (0.09±0.01) (Figure 1). In fact, very few clutches were laid on the pedicels of the leaves, twigs, fruit, and flower buds.

The pattern of distribution of clutches on the studied tree species presented some variations. In the oak trees, the main oviposition site was the edge of the leaves (1.64±0.33), significantly higher than the underside (0.11±0.02) and “others” (0.11±0.04) (Kruskal-Wallis Chi-square, d.f.=3, p=0.0005). The number of clutches laid on the upper side (0.21±0.04) of the leaves was intermediate and not significantly different from the number of clutches in the other positions (Table 3).

In the almond trees, chrysopids laid in a similar proportion in all the sites, only showing differences between the abundance of clutches on the upper side (1.47±0.41) and the extremely scarce clutches in “others” (0.08±0.01) (Kruskal-Wallis Chi-square, d.f.=3, p=0.0009) (Table 3). There were no significant differences among the oviposition sites in the olive and pine trees (Table 3).

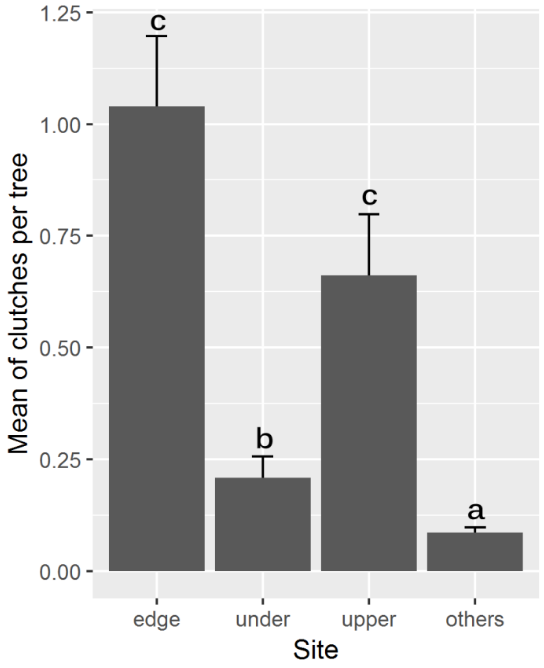

Figure 1. Mean abundance of clutches recorded in each oviposition site (mean±SE) for the total tree sampled. Different letters mean significant differences (Tukey test).

Table 3. Total abundance of clutches per tree species and mean number of clutches recorded per tree in each year, site, and mode of oviposition (mean±SE). Different letters pointed significant differences (Kruskal-Wallis test, Nemenyi post-hoc pairwise comparison: n.s - non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

		2016	2017	Total
Almond (Total clutches)		140	95	235
Oviposition site		n.s	n.s	***
	Upper side	1.2±0.23	1.74±0.85	1.47±0.41^{a}
	Underside	0.35±0.17	0.15±0.08	0.25±0.09^{ab}
	Edge	1.2±0.34	0.31±0.06	0.76±0.25^{ab}
	Others	0.1±0.01	0.06±0.02	0.08±0.01^{b}
Oviposition mode		n.s	n.s	n.s
	Single	0.72±0.17	0.53±0.26	0.62±0.16
	Clusters	0.04	0	0.04
Oak (Total clutches)		90	110	200
Oviposition site		n.s	n.s	***
	Upper side	0.23±0.06	0.18±0.03	0.21±0.04^{ab}
	Underside	0.12±0.03	0.09±0.02	0.11±0.02^{b}
	Edge	1.27±0.45	2.02±0.46	1.64±0.33^{a}
	Others	0.07	0.14±0.06	0.11±0.04^{b}
Oviposition mode		n.s	n.s	n.s
	Single	0.53±0.2	0.71±0.31	0.62±0.18
	Clusters	0.12±0.03	0.1±0.03	0.12±0.02
		2016	2017	Total
Olive (Total clutches)		102	33	135
Oviposition site		n.s	n.s	n.s
	Upper side	1.38±0.17	0.54±0.14	1.04±0.23
	Underside	0.44±0.25	0.18±0.06	0.31±0.13
	Edge	0.42±0.1	0.2±0.04	0.31±0.07
	Others	0.07	0	0.07
Oviposition mode		n.s	n.s	n.s
	Single	0.68±0.18	0.28±0.07	0.5±0.11
	Clusters	0	0	0

Continued Table 3.

Pine (Total clutches)		123	103	226
Oviposition site		n.s	n.s	n.s
	Upper side	0.43±0.12	0.1±0	0.33±0.1
	Underside	0	0	0
	Edge	1.51±0.23	1.24±0.44	1.34±0.28
	Others	0	0.04	0.04
Oviposition mode		n.s	n.s	n.s
	Single	0.96±0.27	1.2±0.47	1.06±0.25
	Clusters	0.46±0.3	0.16±0.06	0.28±0.12

4.4.2. Oviposition mode

In general, single clutches (0.66±0.09) were the most abundant mode of oviposition, showing significant differences compared with cluster clutches (0.17±0.05) (Table 2, Figure 2). In the oak, pine, and almond trees, both modes of oviposition occurred without significant differences in abundance, but only single clutches were observed in the olive trees (Table 3). Group clutches were not observed on any tree.

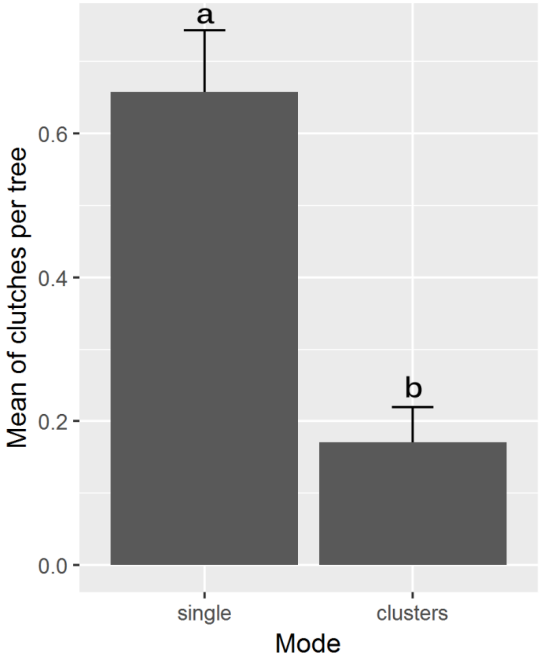

Figure 2. Mean abundance of clutches recorded in each oviposition mode (mean±SE). Different letters mean significant differences (Tukey test).

4.4.3. Lacewing assemblages in olive, almond, oak and pine trees

In total, 455 clutches were collected in August 2016 and, after analysis under stereomicroscope, we found that 5.3% (24 clutches) had been parasitized in the field and the parasites emerged before being collected, showing a particular hole of emergence; 5.5% (25 clutches) had been predated showing the egg cover bitten; 74.3% (338 clutches) in which the chrysopid larvae had hatched previously to collection, and 14.9% (68 clutches) were clutches alive and then raised in the lab.

From these 68 clutches, 121 adult chrysopids emerged which were identified as belonging to four genera and nine different species (Table 4). Most of the adults emerged from clutches in late summer belonged to the *C. carnea* complex (38.2%), *Cunctochrysa baetica* (32.4%) and *P. prasinus* (20.6%). Within the *C. carnea* complex we were able to identify four different species (Table 4), with *Chrysoperla mutata* being the most abundant, which laid single eggs principally on olive, but also in almond and oak trees. *C. mutata* was the only *Chrysoperla* species found laying on olives. *Chrysoperla pallida* and *Chrysoperla lucasina* were found with less abundance only in almond trees, and *C. mediterranea* was found only in pine trees. The species *C. baetica* was widely distributed in all the sampled trees except the pine trees, and it was especially abundant in almond trees. The *P. prasinus* complex was principally made up of *P. prasinus s.str.* which was widely distributed in all the sampled trees. Moreover, we were able to identify the sibling species *P. marianus* only by its cluster clutches, which were frequent in pine trees (Table 4).

Moreover, *P. prasinus* was the only chrysopid species laying clutches on all the sampled trees and the almond was the preferred tree for laying by the highest number of chrysopid species (six species), followed by the oak (five species), pine and olive trees (three species) (Table 4).

As far as to the site of oviposition was concerned, most of the studied chrysopid species laid their clutches in a variety of positions (although predominantly on the upper side and the edges of the leaves as commented in the *Oviposition site* section), except for *P. prasinus* which predominantly laid its clutches on the underside of leaves (Table 3).

Regarding the oviposition mode, most of the collected late summer chrysopid species (eight out of nine) laid only single clutches with *P. marianus* and *P. flavifrons* being the only species to lay cluster clutches. These species may have an important role on the chrysopids population because, although the number of clutches was low (7.3% of the total collected clutches), they might have contributed with a high number of larvae and adults to their habitats (48.0% of the total individuals hatched in the laboratory) (Table 4).

Table 4. Identified chrysopid species and their oviposition traits. The total number of clutches collected and reared until adult emergence and identification is in brackets.

Specie names	No. of clutches	No. of specimens	Oviposition mode	Oviposition sites	Tree species
Chrysopa viridana Schneider, 1845	1	1	single	upper side (1)	almond (1)
Chrysoperla lucasina (Lacroix, 1912)	3	3	single	upper side (1), edge (2)	almond (3)
Chrysoperla mediterranea (Hölzel, 1972)	5	5	single	upper side (2), edge (3)	pine (5)
Chrysoperla mutata (McLachlan, 1898)	12	12	single	upper side (9), edge (2), underside (1)	almond (3), oak (1), olive (8)
Chrysoperla pallida Henry et. al, 2002	6	6	single	upper side (4), underside (1), others(1)	almond (6)
Cunctochrysa baetica (Hölzel, 1972)	22	22	single	upper side (9), edge (11), underside (2)	almond (11), olive (4), oak (7)
Pseudomallada flavifrons (Brauer, 1851)	1	9	cluster	underside (1)	oak (1)
Pseudomallada marianus (Navás, 1905)	4	49	cluster	upper side (4)	oak (1), pine (3)
Pseudomallada prasinus (Burmeister, 1839)	14	14	single	upper side (2), edge (2), underside (10)	almond (5) oak (3), olive (5), pine (1)

4.5. Discussion

In our study, we found a similar mean abundance of clutches per tree on the four studied tree species: olive, almond, oak, and pine trees. This fact points to female chrysopids be able to find the suitable architectural complexity and vegetal structures and/or abundant food for them and their progeny in the entire studied tree species, both factors considered as important for determining oviposition behavior (Canard et al. 1984, Duelli 1984b, Nakamura et al. 2000). Thus, we can conclude that the presence of any tree surrounding the olive groves is favoring the chrysopid abundance and their role as pest predators in the same manner.

Additionally, we found that the abundance of clutches was also stable between the two years that we studied and among the different study locations. This stability maybe promoted by the high ability of chrysopid dispersion and their low specificity for alternative food and suitable sites for oviposition, mating, and overwintering that is offered by the olive groves and the surrounding seminatural vegetation. This vegetation suffers very few changes in a long-term period ensuring the continuity of chrysopid populations and their ecological function (Szentkirályi 2001b).

4.5.1. Oviposition sites

We found that the edges (frequently spiny in Mediterranean vegetation) together with the upper side of the leaves were the most frequently chosen sites by chrysopids to lay their eggs, taking into account all the studied trees as a whole. This is in agreement with previous results that lacewings apparently prefer to lay on hairy plant organs (Szentkirályi 2001a, b). In the selected oviposition sites (edge and upper side of the leaves), the clutches are more exposed to meteorological factors or management labors than on the underside of the leaves. Nevertheless, the canopy of trees provides important shelter against meteorological phenomena (Stelzl and Devetak 1999) and management labours are scarce during late summer both in the olive groves as well as in the other surrounding trees. Moreover, Szentkirályi (2001c) found that the silviculture techniques applied to these habitats have proven beneficial to the chrysopids.

Nevertheless, there was not a clear oviposition site selection pattern in each tree species, except for oak trees in which the general pattern is repeated: the upper sides and edges are the preferred sites. In pine trees, we did not find a clear tendency for laying clutches on the edges or the tips of the leaves, contrary to what had been found in other studies (Duelli 1984a, 1986b, Monserrat 2016). These authors indicated that laying eggs on the tips of the leaves might be associated with an ancient ovipositional behavior linked to small-sized chrysopids. However, the species we identified laying clutches on pine trees in late summer were *C. mediterranea* and *P. prasinus,* which are not small.

4.5.2. Oviposition mode

Our results showed a dominant abundance of single rather than cluster clutches in all the studied trees. In fact, eight chrysopid species laid single clutches and only two (*P. marianus* and *P. flavifrons*) laid cluster clutches. No group clutches were found in our study. Pantaleoni (1996) had a similar result but only in oak trees, where he found that most of the clutches were single and only four cluster clutches were collected, belonging to *P. flavifrons*. To lay single eggs has been stated as a strategy for avoiding predation, the continuous mobility of chrysopids during reproduction allows a better spread of the offspring and by spacing the eggs and young larvae, they have a better chance of evading sibling cannibalism or parasitism. An alternative selective explanation also exists, in which laying eggs in clusters in arboreal species facilitates sibling cannibalism in habitats with intense fluctuations in food supply (Duelli 1984b). Then, the species laying eggs in clusters might have an especially relevant role in periods of intense seasonal perturbations, such as droughts in Mediterranean ecosystems.

4.5.3. Lacewing assemblages in almond, oak, olive and pine trees

Our study provides important information about the oviposition behavior of some of the most problematic species belonging to the genera *Chrysoperla* and *Pseudomallada*. This aspect is crucial and allows us to have a better appreciation of the activity and effect of these important auxiliary insects (Pantaleoni 1996). In our study 10 chrysopid species were found to be active in late summer, laying eggs around and on the olive trees.

Thus, *P. prasinus* is confirmed as the most generalist chrysopid spreading its clutches throughout all the studied trees, and the only one clearly preferring the underside of leaves as their oviposition site. Several authors have also found this species in oaks (Marín and Monserrat 1987, Marín 1994, Pantaleoni 2001, Szentkirályi 2001c) and pines (Czechowska 1994, Marín 1994). In olive trees, *P. prasinus* has been stated as an important predator species due to its abundance and its synchronisation with the preimaginal stages of olive pests (Alrouechdi 1984). In Italian olive groves, all the stages of this species were found

(Pantaleoni 2001). To the best of our knowledge, this is the first time its clutches have been recorded on almond trees.

We collected clutches of *P. marianus* in oak and pine trees, partially agreeing with the results shown by Monserrat (2016) and Szentkirályi (2001b). This species has recently been confirmed as a valid species and segregated from *P. prasinus* (Canard and Thierry 2017); therefore, some already published information about *P. prasinus* may in fact belong to *P. marianus* (Monserrat 2016). The oviposition mode in cluster clutches has been crucial and has allowed us to identify this sibling species.

Moreover, the *C. carnea* complex has also been the most abundant as a whole in our study. Nevertheless we have been able to identify four out seven species pertaining to this complex group in our study, agreeing with Monserrat (2016). *Chrysoperla mutata* turned out to be the only *Chrysoperla* species linked to olive trees in our study, while *C. mediterranea* was linked to pine trees (as previously reported: Marín 1994, Monserrat and Marín 1994) and *C. pallida* and *C. lucasina* were linked to almond trees. This result is much more restricted than that expected according to Monserrat (2016), who recorded the possibility of finding adults of these species in all the studied trees.

Strikingly, all the adults of *C. mediterranea* that hatched in the laboratory were collected as single clutches, in contrast to the statement of Monserrat (2016) who referred to this species laying its eggs as group clutches.

Moreover, *C. baetica* laid its clutches on almond and olive, even in oak trees. In general, it has already been stated that adults of the species of the *Cunctochrysa* genus visited olive (Campos and Ramos 1983) and occasionally oak, pine, and almond trees (Monserrat 2016).

4.6. Conclusion

In conclusion, all the tree species studied are equally suitable to be used for oviposition and the chrysopid population is stable between years and amongst locations. In general, the most abundant species are spread out in all (*P. prasinus*) or most of the trees (*C. baetica*), although some of them appeared only on a determined tree (*C. mediterranea* on pine trees, *C. lucasina* and *C. pallida* on almond trees) and *C. mutata* is the only *Chrysoperla* species found to lay on olive trees.

In consequence, to maintain any of these tree species in the surroundings of the olive groves is an important way of habitat management, making it possible to increase the chrysopid population near the olive crops and to maintain their function as pest predators. Moreover, maintaining a diversity of trees surrounding olive groves also ensures the existence of diverse chrysopid assemblages resilient to periodical perturbations (such as chrysopid laying cluster clutches).

Almond trees, sheltering the most diverse assemblage of chrysopid clutches, have traditionally been cultivated together with olive trees. This ancestral and synergic assemblage, currently less and less utilized, probably provided an important predator diversity which conferred resilience to the olive agro-ecosystem. We encourage farmers to maintain this crop together with olive trees, at least in the surroundings of the groves.

Similar but extended long-term annual studies should help to clarify the laying period and preferences of different species of the genus *Chrysoperla, Pseudomallada and Cunctochrysa* as recommended by other authors (Pantaleoni 2001).

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Abundance and population decline factors of chrysopid juveniles in olive groves and adjacent trees

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5.1. Abstract

Numerous species of the family Chrysopidae, commonly found in agroecosystems, whose larvae predate on several pests of economic importance, are regarded as biological control agents. Their abundance and diversity are influenced by vegetation cover, although little is known about the effects of seminatural habitats on their populations. The objective of this study is to gain a better understanding of the relationship between the trees in seminatural habitats adjacent to olive groves, juvenile stages of the family Chrysopidae and factors influencing their population decline, which is crucial for an effective habitat management program aimed at conserving these important predators. Using cardboard band traps (eight per tree), the juvenile stages were collected from 25 almond, oak, olive and pine trees over a one-year sampling period. The population decline was caused by parasitoids (26.5%), predators (5.1%) and unknown factors (13.2%). In addition, chrysopids established in olive trees showed the lowest rate of parasitism. We identified ten chrysopid species that emerged from the juveniles collected from almond, oak, olive and pine trees, with a predominance of *Pseudomallada prasinus*. The chrysopid-parasitoid complex was composed of five species; *Baryscapus impeditus* (Eulophidae), which was the most abundant, was preferentially associated with *Chrysopa pallens*, *Chrysoperla lucasina* and *Chrysoperla mediterranea*.

Keywords: parasitoids; *Chrysoperla carnea* complex; ecological infrastructures; *Olea europaea*; *Pinus halepensis*; *Prunus dulcis*; *Quercus rotundifolia.*

5.2. Introduction

Of the many families of the Order Neuroptera, Chrysopidae attracted the most attention as compare to Coniopterygidae and Hemerobiidae (Monserrat 2016), as numerous species belonging to the Chrysopidae family are regarded as biological control agents given their potential impact on pest populations in crops (Principi and Canard 1984, New 1986, Duelli 2001, Szentkirályi 2001a, Pappas et al. 2011). Larvae are active polyphagous predators of soft-bodied arthropods, such as aphids, whiteflies, thrips and mites, in addition to being widely distributed in agroecosystems (Principi and Canard 1984, New 1986, Duelli 2001, Szentkirályi 2001a, Pappas et al. 2011).

Chrysopidae is the second most important family in terms of the number and diversity of species with 1,423 valid species belonging to 82 genera (Oswald 2018). *Chrysoperla carnea* (Stephens, 1836) *sensu lato*, which has been reared and released in crops around the world (Alrouechdi 1981, Daane et al. 1996, Gerling et al. 1997, Tauber et al. 1997), is the species most commonly used in agricultural biological control programs (New 1975a). There is evidence that *C. carnea* is a complex of at least 21 cryptic species (Henry 1985, Tauber and Tauber 1986, Monserrat 2016). Although some species are well defined with respect to morphological characteristics, habitats, courtship songs and molecular techniques, their taxonomy has not been fully resolved (Henry et al. 1996, Henry et al. 2002, Canard and Thierry 2007, Henry et al. 2013, Price et al. 2015). A recent review of the green lacewing shows that seven species belong to the *Chrysoperla* Steinmann, 1964 genus in the Iberian Peninsula and Balearic Island (Monserrat 2016).

In previous studies 33 species of the Chrysopidae family were identified in olive groves, with the *Chrysoperla carnea* complex (Stephens, 1836) and the genus *Pseudomallada* Tsukaguchi, 1995 being particularly noteworthy (Campos 1989, Monserrat and Marín 1994, Szentkirályi 2001b, Porcel 2012). The larval stages of these chrysopids are key predators of the three main pests in olive groves: *Prays oleae* (Bernard, 1788), *Saissetia oleae* (Olivier, 1791) and *Euphyllura olivina* (Costa, 1839) (Alrouechdi 1980, Ramos et al. 1984, Campos 1989, Szentkirályi 2001a). The use of green lacewings to improve biological pest control in olive groves has been evaluated (Campos 2001). McEwen et al. (1994) attempted to attract *C. carnea* by spraying artificial honeydew (McEwen et al. 1994), and another study has shown that a relationship exists between non-crop vegetation and green lacewing oviposition in olive groves (McEwen and Ruiz 1994). Porcel et al. (2017) also found that resident vegetation cover has a positive effect on green lacewings abundance and diversity in olive groves. However, the role of seminatural habitats adjacent (bordering and around) to olive groves is poorly understood.

Chrysopid populations are regulated by predation (intraguild and cannibalism) and parasitism which are particularly harmful (Canard et al. 1984, McEwen et al. 2001), and their development is also affected by abiotic conditions such as temperature, humidity and day length (Chang et al. 1995, Canard and Volkovich 2001, Pappas et al. 2008, Pappas et al. 2013, Nadeem et al. 2014). In fact, the eggs and larvae of *C. carnea s.l.* are attacked and killed by coccinellids, reduvids, carabids, spiders and ants (Vinson and Scarborough 1989, Cisneros and Rosenheim 1997, Lucas et al. 1997, Dinter 1998, Morris et al. 1998), as well as by cannibalistic individuals from its own species (Canard and Duelli 1984). The chrysopid-parasitoid complex is composed of species from the Orders Hymenoptera and Diptera, in addition to mites, fungae and certain viruses, which can affect all stages of chrysopid development, ranging from the egg and larva stages to adulthood (Killington 1932, Clancy 1946, Sidor 1960, Alrouechdi and Panis 1981, Canard et al. 1984, Ventura et al. 2000 (2001)); some genera of the Order Hymenoptera, such as *Isodromus* Howard, 1887, *Baryscapus* Förster, 1856, *Helorus* Panzer, 1798 and *Gelis* Thunberg, 1827 are primary parasitoids of Chrysopidae, while others, such as *Perilampus* Latreille, 1809, *Dichrogaster* Doumerc, 1855, *Pteromalus* Swederus, 1795 and *Eupelmus* Dalman, 1820, are primary parasitoids of Chrysopidae and hyperparasitoids (Principi 1948, Judd 1949, Muma 1959, Mehra 1966, New 1967b, a, Ickert 1968, New 1975b, Alrouechdi et al. 1981, Alrouechdi and Panis 1981, New 1982, Alrouechdi et al. 1984). Other factors affecting larval mortality include abiotic conditions and the food resource availability. The impact of all these factors can vary according to the species of chrysopid and its habitat, which need to be accurately characterized when biological control is planned both for conservation purposes and through mass release of chrysopids (Daane 2001).

Faced with natural enemies, chrysopids have developed defensive strategies and behaviours, such as nocturnal and twilight activity, cryptic, aposematic and disruptive coloration (Withycombe 1923, Monserrat 2015), stalked eggs (Smith 1926, Duelli 1984, 1986), thanatosis (New 1986), as well as segregation of foul-smelling substances produced by adults and toxic, crippling and disruptive substances secreted by larvae (Kennett 1948, Blum et al. 1973, Rothschild et al. 1973, Monserrat 1980, New 1986, Güsten and Dettner 1991, Monserrat 2015). It has also been suggested that exogenous material on the backs of larvae of certain chrysopid genera (*Pseudomallada* and *Rexa* Navás, 1920) could act as a physical barrier against predators and parasitoids (Smith 1921, 1926, Kennett 1948, New 1969, Eisner and Eisner 2002, Monserrat and Diaz-Aranda 2012).

Given the generalist predatory behaviour and dispersive capacity of chrysopids, their populations in olive groves are influenced by the vegetation and natural habitats adjacent to the crop, where they can find alternative prey, pollen, nectar, as well as reproduction and refuge sites. Thus, spontaneous vegetation cover between the rows of olive trees has been reported to increase chrysopid abundance and diversity in the crop (Porcel et al. 2017). Additionally, tree species such as *Quercus rotundifolia* L., *Pinus halepensis* (Mill.) and *Prunus dulcis* D.A. Webb, which are an integral part of the olive grove landscape in Spain, are visited by chrysopids (Monserrat and Marín 1994, Szentkirályi 2001a, González et al. 2008) and used as oviposition sites by different species (Alcalá Herrera et al. 2019). Studies of their population dynamics in olive groves should therefore include the effect of adjacent vegetation, as research on chrysopid parasitism has, up to now, focused on different arboreal species and crops while neglecting activity in the surrounding landscape (Putman 1937, Clancy 1946, Principi 1947, 1948, Judd 1949, Principi 1956, Muma 1959, Alrouechdi and Panis 1981, Campos 1986).

This study aims to assess the relationship between trees in seminatural habitats adjacent to olive groves, the juvenile stages of the family Chrysopidae and population decline factors (parasitism, predation and unknown factors).

We expected (a) to collect chrysopid juveniles from all the tree species studied, from which adult chrysopids had previously been sampled (Monserrat and Marín 1994), and (b) to record a medium to high chrysopid parasitism rate in olive trees which was predicted to be similar in all three trees species (almond, oak and pine) (Neuenschwander and Michelakis 1980, Alrouechdi et al. 1981, Campos 1986). Finally, as we expected the chrysopids to be parasitized, we studied the relationship between parasitoid and chrysopid assemblages while taking into account the season and tree species (almond, oak, olive and pine) in which the interaction occurred.

The knowledge acquired is a crucial prerequisite for an effective habitat management program aimed at conserving the populations of these important predators.

5.3. Materials and Methods

5.3.1. Area of study

The study was carried out in the Montes Orientales region, 20 km to the north of the Andalusian province of Granada, which is the fourth largest area devoted to olive grove crops, covering 198,331 hectares (ha) (Junta de Andalucía 2015). The landscape in this region is dominated by olive plantations, with patches of semi-natural vegetation mostly composed of *P. halepensis*, *Q. rotundifolia* and *P. dulcis*, in addition to less abundant species, such as *Quercus coccifera* L. (Fagales: Fagaceae), *Juniperus oxycedrus* L. (Pinales: Cupressaceae), *Cistus albidus* L. (Malvales: Cistaceae), *Cistus clusii* Dunal (Malvales: Cistaceae), *Genista cinerea* (Vill.) DC. (Fabales: Fabaceae), *Lavandula latifolia* Medik. (Lamiales: Lamiaceae), *Pistacia terebinthus* L. (Sapindales: Anacardiaceae), *Rosmarinus officinalis* L. (Lamiales: Lamiaceae), *Thymus mastichina* (L.) L. subsp. *mastichina* (Lamiales: Lamiaceae), *Thymus zygis* L. subsp. *gracilis* (Boiss) R. Morales (Lamiales: Lamiaceae) and *Ulex parviflorus* Pourr. (Fabales: Fabaceae).

Sampling was carried out in five organic olive farms (Table 1) in conformity with EU legislation (European Union 2007, 2008). All these farms are located at a similar altitude of 800 to 1,100 meters above sea level, the variety of *Olea europaea* L. is “Picual” and the plantation schemes are very similar (8x8 and 12x12 meters), with areas ranging from 0.9 to 215 ha. Soil management practices on these farms include the maintenance of spontaneous vegetation cover, which is eliminated by mechanical mowing and/or grazing between April and May. In addition, during the post-harvest period, the soil is fertilized with organic matter, and crushed pruning waste is placed in the rows between crops to create inert cover. The incidence of disease (such as *Fusicladium oleagineum*) and pests (such as *P. oleae* and *Bactrocera oleae* (Gmelin, 1790)) was remedied by timely and targeted treatment (two aimed at diseases and one for pests) using products listed in Annex II of Commission Regulation (EC) no. 889/2008.

Table 1. Characteristics and availability of each tree species and number of tree species sampled in each sampling site per month sampled.

Site	Coordinates	Area (ha)	Number of trees sampled				
			Almond	Oak	Olive	Pine	Total
Norberto	37°19'5.96"N; 3°34'9.92"W	4.3	9	5	5	9	28
La Pedriza	37°20'17.44"N; 3°33'39.21"W	0.9	-	5	5	8	18
Los Almendros	37°22'24.76"N; 3°37'46.03"W	215	8	5	5	-	18
Píñar (right)	37°24'14.29"N; 3°29'14.13"W	58	-	5	5	8	18
Píñar (left)	37°24'40.93"N; 3°28'52.41"W	124	8	5	5	-	18
Total			25	25	25	25	100

5.3.2. Collection of samples

To collect the juvenile stages of chrysopids (larvae and prepupae/pupae), eight corrugated cardboard band traps (10x17.5 cm) were placed in a total of 100 trees (25 trees per species): *O. europaea* (olive), *Q. rotundifolia* (oak), *P. dulcis* (almond) and *P. halepensis* (pine), whose distribution in the sampling sites depended on their availability in the study area (Table 1). The band traps were installed on different branches located 160-170 centimetres from the ground taking into account the four cardinal directions (two band traps per direction). The 800 band traps were changed each month between June 2016 and May 2017 (a total of 12 sampling events) on the same 100 trees (identified by number).

In the laboratory, the juvenile stages -larvae, “open cocoons”, with one or more apertures caused by the emergence of chrysopid or parasitoid adults and predators feeding on juveniles, as well as “closed cocoons”, with no apertures and containing a chrysopid larva- were individually labelled and kept in Petri dishes (55 mm in diameter) for observation and monitoring. The trash-bearing juveniles (with exogenous material on their backs) and naked juveniles (with no exogenous material) were also quantified. The larval instars and “closed cocoons” were kept in an incubation chamber (Fitoclima S600 PLH; Aralab, Rio de Mouro, Portugal) in order to monitor their development at a temperature of 25±1°C, a humidity of 50-60% and a photoperiod of 16:8 (L:D) hours.

The individual larvae were fed *ad libitum* with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (EphestiaTop; Biotop; Livron-sur-Drôme; France) to facilitate the completion of their biological cycle up to the adult stage and taxonomic identification.

The juveniles that failed to reach the adult stage were inspected under a stereomicroscope (Nikon SMZ 800; Nikon, Tokyo, Japan) in order to ascertain whether death was due to parasitoids or unknown factors. Additionally, we determined whether the aperture in the “open cocoons” was caused by the emergence of an adult chrysopid, a parasitoid or by the feeding of predators. In parasitized cocoons, the number of emerged adult parasitoids, as well as the number and average diameter of exit apertures were quantified (Figure 1).

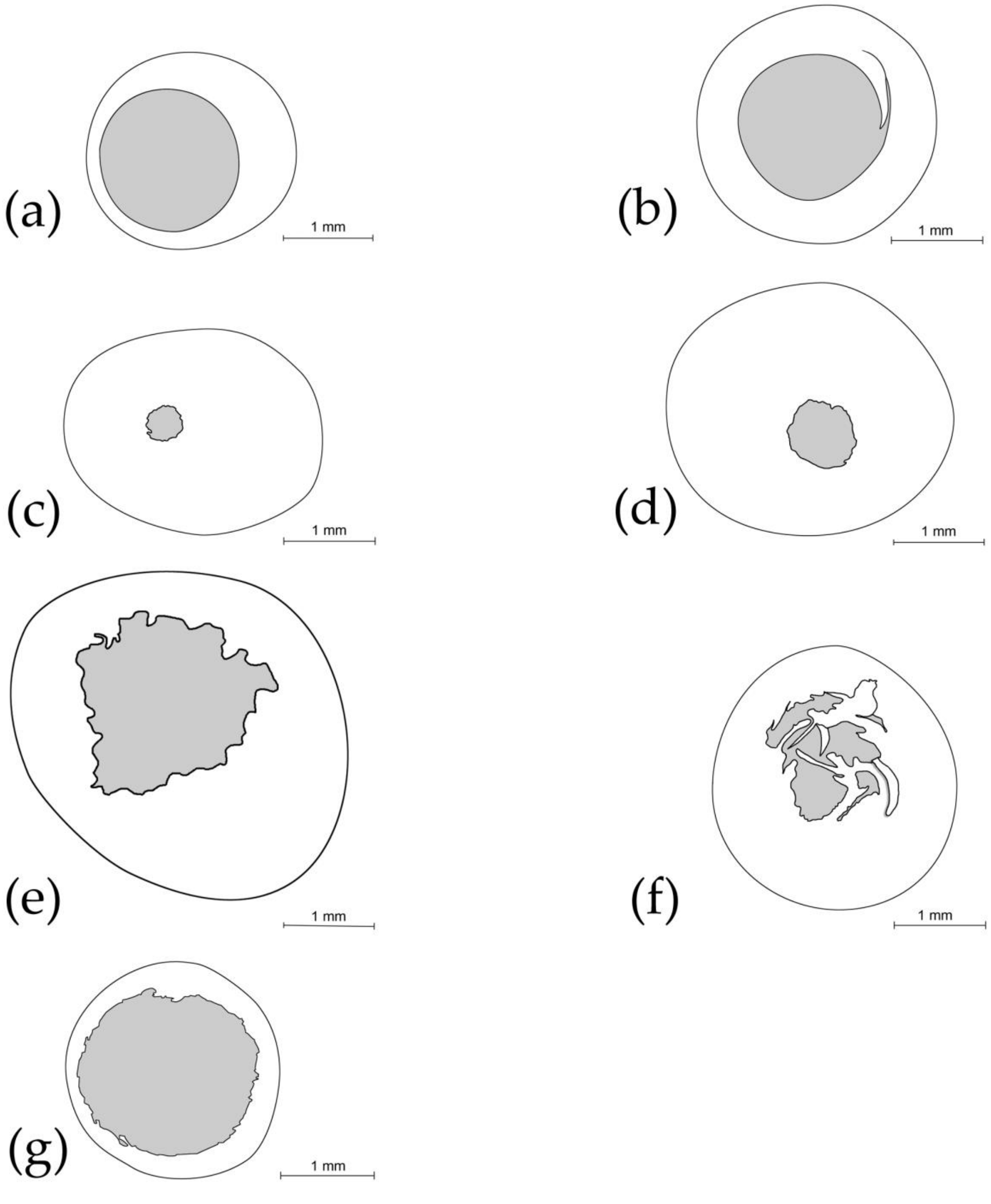

Figure 1. Examples of apertures in cocoons made by (a) the Chrysopidae family, by the parasitoid complex: (b) *H. ruficornis*, (c) *B. impeditus,* (d) *I. puncticeps*, (e) *P. minutalis*, (f) *G. ilicicola* and by (g) predators.

The adult chrysopids that emerged in the laboratory were identified taxonomically up to species level according to the Monserrat key (2016). The emerged adult parasitoids in the laboratory were identified up to species level with the aid of taxonomists with specialist knowledge of the different families (see acknowledgements), the Plant Protection Group collection at the Estación Experimental del Zaidín (EEZ) and the Goulet and Huber key (1993).

5.3.3. Statistical analysis

All analyses were carried out using R software version 3.5.0 (R Development Core Team 2017).

Statistical analysis began with data exploration (Zuur et al. 2010). We explored the total abundance of the juvenile stages collected in four categories (adult, parasitized, and predated chrysopids; unknown factors) in the tree species sampled throughout the study period. For data presentation purposes, the study period was simplified by grouping the sampling dates by season: summer (June, July and August), autumn (September, October and November), winter (December, January and February) and spring (March, April and May). Juveniles (from larvae and "open or closed cocoons"), which produced an adult chrysopid and emerged either in the laboratory or in the field, were categorized under the heading "adult chrysopids". A similar system was used for parasitoids from juveniles, which were grouped under the heading "parasitized chrysopids". Death of juveniles caused by other population decline factors were classified as "unknown factors". Finally, "open cocoons" with apertures due to attacks by predators, were defined as "predated chrysopids".

We then analysed the total abundance of juvenile stages collected from each tree species sampled using a generalized linear mixed model (GLMM) with a negative binomial distribution (formula 1 to 3) and a log link function (formula 4) in relation to tree species, site and month sampled as fixed factors and the identification of the individual tree as the random factor (formula 4 and 5) using the "lme4" software package (Bates et al. 2014):

$$\text{Abundance of juvenile stages} \sim \text{NB}(\mu_{ij}, \text{k}) \tag{1}$$

$$\text{E(Abundance of juvenile stages}_{ij}) = \mu_{ij} \tag{2}$$

$$\text{var(Abundance of juvenile stages}_{ij}) = \mu_{ij} + \frac{\mu_{ij}^2}{k} \tag{3}$$

$$\text{Log}(\mu_{ij}) = \text{tree species}_{ij} + \text{site}_{ij} + \text{month sampled}_{ij} + a_j \tag{4}$$

$$a_j \sim \text{N}(0, \sigma^2 \text{individual tree}) \tag{5}$$

We then calculated the rate of parasitism per tree (%) expressed as the number of juvenile stages affected by parasitism in each tree divided by the total number of juvenile stages collected from each tree multiplied by 100. The rate of parasitism was analysed with the aid of the GLMM with a binomial distribution (formula 6) and a logit link function (formula 7) using tree species, site and month sampled as fixed factors and the identification of the individual tree as the random factor (formula 7 and 8). The “lme4” software package was used for this analysis (Bates et al. 2014):

$$\text{Parasitism rate}_{ij} \sim \text{Bin}(1, p_{ij}) \tag{6}$$

$$\text{Logit}(p_{ij}) = \alpha + \beta_1 \text{ x Tree species}_{ij} + \beta_2 \text{ x site}_{ij} + \beta_3 \text{ x month sampled}_{ij} + a_j \tag{7}$$

$$a_j \sim N(0, \sigma^2 \text{individual tree}) \tag{8}$$

The models were constructed and selected according to Akaike Information Criteria (AIC) (Burnham and Anderson 2002). We also analysed the model residuals and checked for uniformity using the “DHARMA” software package (Hartig 2018). The multiple comparisons in each model (chrysopid abundance and parasitism rate) for the tree species, site and month sampled variables were checked with the aid of the post-hoc Tukey test using the “multcomp” software package (Hothorn et al. 2008).

The data for juveniles categorized as “unknown factors”, “predated chrysopids” and “adult chrysopids” were analysed by applying the Kruskal-Wallis test with a Bonferroni adjustment with the aid of the “agricolae” software package (De Mendiburu 2014).

In addition, we calculated the parasitism rate according to the trash-bearing and naked juveniles collected. The rate of parasitism was analysed by applying the Kruskal-Wallis test with a Bonferroni adjustment with the aid of the “agricolae” software package (De Mendiburu 2014).

We employed redundancy analysis (RDA) to determine whether a relationship exists between the composition of chrysopid and parasitoid species and environmental variables (tree species and season). The results were presented using a triplot correlation with the aid of the “vegan” software package (Oksanen et al. 2018)

5.4. Results

5.4.1. Analysis of collected cocoons

We separated the “open cocoons” from “closed cocoons”. “Open cocoons” were classified as “adult chrysopids” (Figure 1a) which emerged from a single circular orifice with a regular border and an average diameter of 1.65±0.01 mm (n=5 cocoon apertures). Parasitized juveniles were classified as “parasitized chrysopids” (Figure 1b, c, d, e, f) which emerged through one, two or three regular or irregular circular apertures with a diameter ranging from 0.4 to 1.7 mm (n=15 cocoon apertures), with the remains of the juvenile host still inside the cocoon. “Open cocoons” were also classified as “predated chrysopids”, with one or two even or uneven circular apertures with an average diameter of 1.7±0.07 mm (n=5 cocoon apertures) (Figure 1g) to feed on juveniles stages, without remains of the juvenile host inside the cocoon. “Closed cocoons” contain prepupa or pupa which can emerge as “adult chrysopids”, can become “parasitized chrysopids” or may not emerge at all and die due to “unknown factors”.

A total of 1,345 juvenile stages of chrysopids were collected between June 2016 and May 2017, over half of which (741 juveniles; n=1,200 trees sampled) completed their development to adulthood in the laboratory or in the field. The other juveniles (604 juveniles; n=1,200 trees sampled) failed to reach adulthood due to the action of parasitoids (357 juveniles; n=1,200 trees sampled), predators (69 juveniles; n=1,200 trees sampled) and unknown factors (178 juveniles; n=1,200 trees sampled) (Table 2).

Table 2. Abundance (%) and categories of juvenile stages in almond, oak, olive and pine trees by season.

Season	Tree species	Adult chrysopids	Parasitized chrysopids	Predated chrysopids	Unknown factors	Total
Summer	Almond	122 (36.7)	144 (43.4)	33 (9.9)	33 (9.9)	332
	Oak	49 (48)	34 (33.3)	9 (8.8)	10 (9.8)	102
	Olive	130 (76.9)	9 (5.3)	8 (4.7)	22 (13)	169
	Pine	109 (63.7)	34 (19.9)	3 (1.8)	25 (14.6)	171
	Subtotal	410 (53)	221 (28.6)	53 (6.8)	90 (11.6)	774
Autumn	Almond	67 (45.3)	54 (36.5)	6 (4.1)	21 (14.2)	148
	Oak	43 (55.8)	18 (23.4)	3 (3.9)	13 (16.9)	77
	Olive	77 (72.6)	11 (10.4)	4 (3.8)	14 (13.2)	106
	Pine	18 (42.9)	19 (45.2)	2 (4.8)	3 (7.1)	42
	Subtotal	205 (55)	102 (27.3)	15 (4)	51 (13.7)	373
Winter	Almond	13 (68.4)	0 (0)	0 (0)	6 (31.6)	19
	Oak	3 (18.8)	11 (68.8)	0 (0)	2 (12.5)	16
	Olive	24 (72.7)	1 (3)	0 (0)	8 (24.2)	33
	Pine	0 (0)	0 (0)	0 (0)	0 (0)	0
	Subtotal	40 (58.8)	12 (17.6)	0 (0)	16 (23.5)	68
Spring	Almond	23 (76.7)	1 (3.3)	0 (0)	6 (20)	30
	Oak	26 (65)	11 (27.5)	0 (0)	3 (7.5)	40
	Olive	29 (60.4)	7 (14.6)	1 (2.1)	11 (22.9)	48
	Pine	8 (66.7)	3 (25)	0 (0)	1 (8.3)	12
	Subtotal	86 (66.2)	22 (16.9)	1 (0.8)	21 (16.2)	130
Total		741 (55.1)	357 (26.5)	69 (5.1)	178 (13.2)	1,345

5.4.2. Abundance and identification of chrysopids

The abundance of chrysopids fluctuated during all four seasons. According to the results of the GLMM (Table 3, Table S1), the summer months showed by far the greatest abundance of chrysopids per tree (2.58±0.28; 774 juveniles; n=300 trees sampled), while the winter months recorded the lowest abundance (0.23±0.04; 68 juveniles; n=300 trees sampled) (Table 2). The months of autumn (1.24±0.14; 373 juveniles; n=300 trees sampled) and spring (0.43±0.06; 130 juveniles; n=300 trees sampled) registered intermediate values. In the spring period, the abundance of juveniles in May (0.82±0.16; n=100 trees sampled) was higher than that in all the winter months: December (0.18±0.05; n=100 trees sampled), January (0.16±0.05; n=100 trees sampled) and February (0.33±0.08; n=100 trees sampled) (Table 3, Table S1).

Chrysopid abundance varied significantly between sites according to the GLMM (Table 3, Table S1); the Norberto farm presented the highest abundance (2.04±0.25; n=336 trees sampled) as compared to the other sites, while the Píñar farm (left) had the lowest abundance (0.47±0.09; n=216 trees sampled); the other sites (Los Almendros, Píñar farm (right) and La Pedriza) reported intermediate values and significant inter-site differences (Table 3, Table S1).

Tree species was also a variable factor in the abundance of juvenile stages of chrysopids (Table 3, Table S1). Pine trees exhibited significantly lower abundance of juveniles per tree (0.75±0.13; 225 juveniles; n=300 trees sampled) as compared to the other tree species: almond (1.76±0.27; 529 juveniles; n=300 trees sampled), olive (1.19±0.13; 356 juveniles; n=300 trees sampled) and oak (0.78±0.08; 235 juveniles; n=300 trees sampled), with no significant differences being observed between the latter three species (Table 3, Table S1).

Table 3. Anova (type II Wald Chi-square test) results of GLMM models (chrysopid abundance and parasitism rate). Significance codes: ****P* <0.001, ***P* <0.01, **P* <0.05.

Model	**Variable**	**χ^2**	**Degree of freedom**	***p* value**	
Chrysopid abundance	Tree species	29.168	3	< 0.001	***
	Site	48.165	4	< 0.001	***
	Month sampled	320.795	11	< 0.001	***
Parasitism rate	Tree species	34.707	3	< 0.001	***
	Site	11.832	4	0.0187	*
	Month sampled	57.895	11	< 0.001	***

The number of juveniles that completed their development to adulthood was by far the highest for those sampled from olive trees (0.87±0.1; 225 juveniles; n=300 trees sampled) (Kruskal-Wallis Chisq=28.57, d.f.=3, *p*<0.001) and lowest in oak trees (0.4±0.05; 121

juveniles; n=300 trees sampled), with almond and pine trees recording intermediate values and with no significant differences between almond, oak and pine trees (Table 2). The number of juveniles killed by "unknown factors" was significantly higher in almond trees (0.22±0.03; 66 juveniles; n=300 trees sampled) than in oak (0.09±0.02; 28 juveniles; n=300 trees sampled) and pine trees (0.09±0.03; 29 juveniles; n=300 trees sampled) (Kruskal-Wallis Chisq 22.79, d.f.=3, *p*<0.001), while no significant differences were observed between almond, oak and pine trees, on the one hand, and olive trees (0.18±0.04; 55 juveniles; n=300 trees sampled), on the other (Table 2). Moreover, the number of "predated chrysopids" in all tree species studied did not differ significantly (Kruskal-Wallis Chisq 5.33, d.f.=3, *p*=0.15).

With regard to temporal distribution, the number of juveniles killed by "unknown factors" collected in summer (0.3±0.05; 90 juveniles; n=300 trees sampled) and autumn (0.17±0.03; 51 juveniles; n=300 trees sampled) was significantly higher than in spring (0.07±0.03; 21 juveniles; n=300 trees sampled) and winter (0.05±0.02; 16 juveniles; n=300 trees sampled), although no significant inter-seasonal differences were observed (Kruskal-Wallis Chisq=49.72, d.f.=3, *p*<0.001). The number of juveniles reaching adulthood was significantly higher in summer (1.37±0.14; 410 juveniles; n=300 trees sampled), followed by autumn (0.68±0.08; 205 juveniles; n=300 trees sampled), spring (0.29±0.04; 86 juveniles; n=300 trees sampled) and winter (0.13±0.03; 40 juveniles; n=300 trees sampled), with significant differences being observed between these last three seasons (Kruskal-Wallis Chisq=126.1, d.f.=3, *p*<0.001) (Table 2).

A total of 440 adult chrysopids belonging to ten species from five different genera of the family Chrysopidae emerged in the laboratory: *Chrysopa* Leach, 1815 (1), *Chrysoperla* (4), *Cunctochrysa* Hölzel, 1972 (1), *Pseudomallada* (3) and *Rexa* (1) (Table 4).

Pseudomallada prasinus (Burmeister, 1839) was the most abundant species (242 individuals) followed by *Chrysoperla pallida* Henry et al., 2002 (74 individuals) and *Chrysoperla mediterranea* (Hölzel, 1972) (63 individuals). The other species were much less numerous: *Chrysoperla lucasina* (Lacroix, 1912) (16), *Chrysoperla mutata* (McLachlan, 1898) (15), *Rexa almerai* (Navás, 1919) (10), *Pseudomallada picteti* (McLachlan, 1880) (7), *Pseudomallada flavifrons* (Brauer, 1851) (5), *Chrysopa pallens* (Rambur, 1838) (4) and *Cunctochrysa baetica* (Hölzel, 1972) (4).

Table 4. Abundance (mean±SE) of chrysopid species that emerged in laboratory from chrysopid juveniles collected from almond, oak, olive and pine trees by season.

Season	Tree species	*Cunctochrysa baetica*	*Chrysoperla lucasina*	*Chrysoperla mediterranea*	*Chrysoperla mutata*	*Chrysoperla pallida*	*Chrysopa pallens*	*Pseudomallada flavifrons*	*Pseudomallada picteti*	*Pseudomallada prasinus*	*Rexa almerai*
Summer	**Almond**	*	0.04±0.02	0	0.04±0.02	0.36±0.09	0.04±0.02	0	*	0.41±0.13	0
	Oak	*	*	0	0.04±0.02	0.07±0.03	0	0	0	0.32±0.08	0
	Olive	0	0.05±0.03	*	0.05±0.03	0.28±0.1	0	0	0	0.12±0.04	0.05±0.03
	Pine	0	0.03±0.02	0,52±0,25	0	0	0	0	0	0	0
Autumn	**Almond**	0	0	*	0	*	0	0	0	0.45±0.14	0
	Oak	0.03±0.02	0	0	*	0	0	0	*	0.17±0.05	0
	Olive	0	0	0	0.04±0.02	0.12±0.04	0	0	*	0.52±0.1	0
	Pin	0	0	0	0	0	0	0	*	*	0
Winter	**Almond**	0	0	0	0	0	0	0	0	0.15±0.05	0
	Oak	0	0	0	0	0	0	*	0	0.09±0.05	0
	Olive	0	0	0	*	*	0	0	0	0.25±0.08	0
	Pine	0	0	0	0	0	0	0	0	*	0
Spring	**Almond**	0	0.08±0.04	0	0	*	*	0	0	0.25±0.1	0
	Oak	0	0	0	0	0.07±0.03	0	0.04±0.02	0.03±0.02	0.13±0.04	0
	Olive	0	0	0	0	0.05±0.04	0	0	0	0.31±0.08	0.08±0.06
	Pine	0	0	0.29±0.12	0	0	0	*	*	*	0

*Mean±SE ≤0.01±0.01.

5.4.3. Parasitism rate and juvenile chrysopid parasitoid complex

The rate of parasitism differed significantly in the arboreal stratum (Table 3, Table S2); the rate for olive trees (4.2±1%; 28 parasitized juveniles; n=300 trees sampled) was significantly below that for the other tree species: almond trees (7.53±1.23%; 199 parasitized juveniles; n=300 trees sampled), oak trees (11.96±1.66%; 74 parasitized juveniles; n=300 trees sampled) and pine trees (6.54±1.29%; 56 parasitized juveniles; n=300 trees sampled); almond, oak and pine trees did not show any significant inter-species differences (Table 3, Table S2).

With regard to the temporal evolution of the parasitism rate, juvenile chrysopids collected in almond trees were found to be affected by parasitism between the months of July and September, reaching a maximum of 34.8% in August. A similar tendency was detected in pine trees, with a maximum of 26.5% recorded in August. On the other hand, juvenile chrysopids in olive and oak trees were affected by parasitism virtually throughout the whole period of the study, with oak trees displaying a maximum rate of 28% in January (Figure 2).

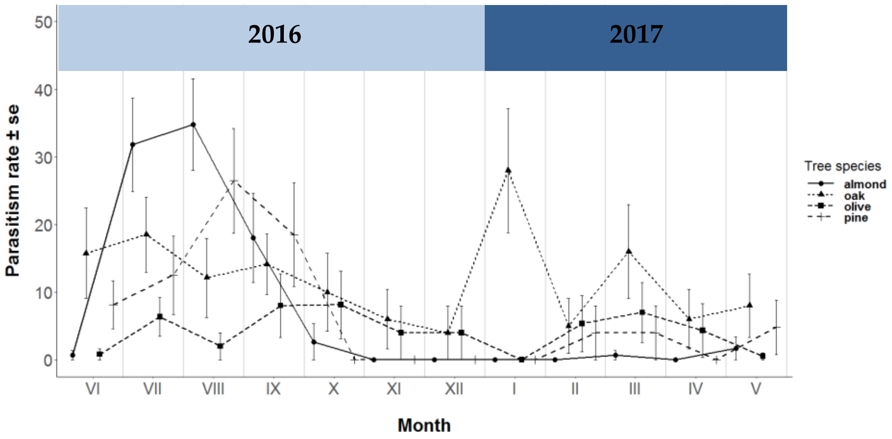

Figure 2. Temporal evolution of parasitism rate (%) in almond, oak, olive and pine trees by month sampled.

With respect to the sites sampled, the average rate of parasitism was found to be significantly higher in the Los Almendros farm (12.24±2%; n=216 trees sampled) as compared to the Norberto farm (8.49±1.23%; n=336 trees sampled), although differences in relation to the other farms (Píñar (right), La Pedriza and Píñar (left)) or with respect to inter-farm rates were not significant (Table 3, Table S2).

On the other hand, the parasitism rate of naked juveniles (5.08±0.55%; 287 juveniles; n=1,200 trees sampled) was significantly higher than that for trash-bearing juveniles

(3.69±0.51%; 70 juveniles; n=1,200 trees sampled) (Kruskal-Wallis Chisq=11.64, d.f.=1, $p<0.001$).

A total of 1,033 parasitoids belonging to five species from five different families of the Order Hymenoptera emerged in the laboratory from 174 parasitized juveniles: *Baryscapus impeditus* (Nees, 1834) (Chalcidoidea: Eulophidae), *Gelis ilicicola* (Seyrig, 1927) (Ichneumonoidea: Ichneumonidae), *Helorus ruficornis* Förster, 1856 (Proctotrupoidea: Heloridae), *Isodromus puncticeps* (Howard, 1885) (Chalcidoidea: Encyrtidae) and *Perilampus minutalis* Steffan, 1952 (Chalcidoidea: Perilampidae) (Table 5).

B. impeditus was the most numerous species (903 individuals from 84 parasitized juveniles). The number of parasitoids per parasitized juvenile ranged from one to 30 (10.75±0.65; n=84 parasitized juveniles), which emerged through one, two or three unevenly edged circular apertures with an average diameter of 0.42±0.02 mm (n=5 cocoon apertures) (Figure 1c). *H. ruficornis* was the second most abundant species (64 individuals from 64 parasitized chrysopids). A single parasitoid emerged from each cocoon through a single helicoidal-shaped aperture with a clearly defined edge and an average diameter of 1.72±0.04 mm (n=5 cocoon apertures) (Figure 1b). With respect to *I. puncticeps* (52 individuals from 12 parasitized chrysopids), the number of individuals per parasitized chrysopid, which emerged, through a single unevenly edged circular aperture with an average diameter of 0.77±0.04 mm (n=5 cocoon apertures), ranged from one to ten (4.33±0.85; n=12 parasitized juveniles) (Figure 1d). The following species were much less abundant: nine *G. iliciola* (Figure 1f) and five *P. minutalis* (Figure 1e) individuals emerged through an unevenly edged aperture with a diameter of 1.11±0.05 mm (n=5 cocoon apertures) and 1.58±0.26 mm (n=5 cocoon apertures), respectively; in both species, each parasitoid emerged from a single parasitized juvenile.

Table 5. Abundance of juvenile chrysopids parasitized (mean±SE) by the parasitoid species complex in almond, oak, olive and pine trees by season.

Season	Tree Species	Juvenile chrysopids parasitized by				
		Baryscapus impeditus	***Gelis ilicicola***	***Helorus ruficornis***	***Isodromus puncticeps***	***Perilampus minutalis***
Summer	**Almond**	0.88±0.23	0.03±0.02	0	0.03±0.02	0
	Oak	0	0.05±0.03	0.16±0.05	*	0
	Olive	0	*	*	*	0
	Pine	0.15±0.05	0	0.11±0.05	0	0
Autumn	**Almond**	*	0	*	*	0
	Oak	0	0	0.17±0.05	0	0
	Olive	*	0	0.07±0.04	0	0.03±0.02
	Pine	0.05±0.04	0	0	0	0
Winter	**Almond**	0	0	0	0	0
	Oak	0	0	0.15±0.05	0	0
	Olive	0	0	0.04±0.02	0	0
	Pine	0	*	0	0	0
Spring	**Almond**	0	0	0	0.04±0.04	*
	Oak	*	0	0.09±0.04	0.04±0.02	*
	Olive	0	*	0.04±0.02	0	*
	Pine	0	0	0	*	0

*Mean±SE ≤0.01±0.01.

5.4.4. Multivariate analysis of the relationship between parasitoid and chrysopid species, tree species and season

Using RDA analysis, we determined that tree species and season accounted for 14.1% of the variation in the parasitoid and chrysopid community. The first two RDA axes accounted for 79% of this variation and adjusted R^2 for 12.8%, suggesting that other variables were not captured by the model.

The RDA correlation triplot (Figure 3) showed that three groups of species were positively inter-correlated. The first group was composed of three chrysopids (*C. baetica*, *P. flavifrons* and *P. picteti*) and one parasitoid (*H. ruficornis*). The abundance of *C. baetica* reached maximum levels in oak trees in autumn, with a similar pattern being observed for *P. flavifrons* and *P. picteti* only in spring, while the parasitoid *H. ruficornis* recorded maximum abundance in oak trees in all seasons (Table 4 and 5).

The second group was composed of three chrysopids (*C. pallens*, *C. lucasina* and *C. mediterranea*) collected in spring and summer and two parasitoids (*B. impeditus* and *I. puncticeps*) (Figure 3). *C. lucasina* appeared in spring in almond trees and then spread to the four tree species, while *C. pallens* was only detected in almond trees, and *C. mediterranea* reached maximum abundance in pine trees in summer (Table 4 and 5). *B.*

impeditus was mainly observed in almond trees and dispersed to pine trees in summer, though with a lower level of abundance, while the other parasitoid species *I. puncticeps* appeared in spring in almond and pine trees and had a preference for almond trees in summer (Table 4 and 5).

The third group is composed of *C. pallida, R. almerai*, *C. mutata*, *P. prasinus* and the parasitoid *P. minutalis*. *R. almerai* only appeared in olive trees in spring and summer, while *C. pallida* was reported in olive trees throughout the year, reaching maximum levels in almond trees in summer. *C. mutata* was mainly recorded in summer and autumn. Finally, *P. prasinus*, though collected from olive and almond trees throughout the year, reached maximum abundance in olive trees in autumn, with the parasitoid *P. minutalis* showing a similar pattern (Tables 4 and 5).

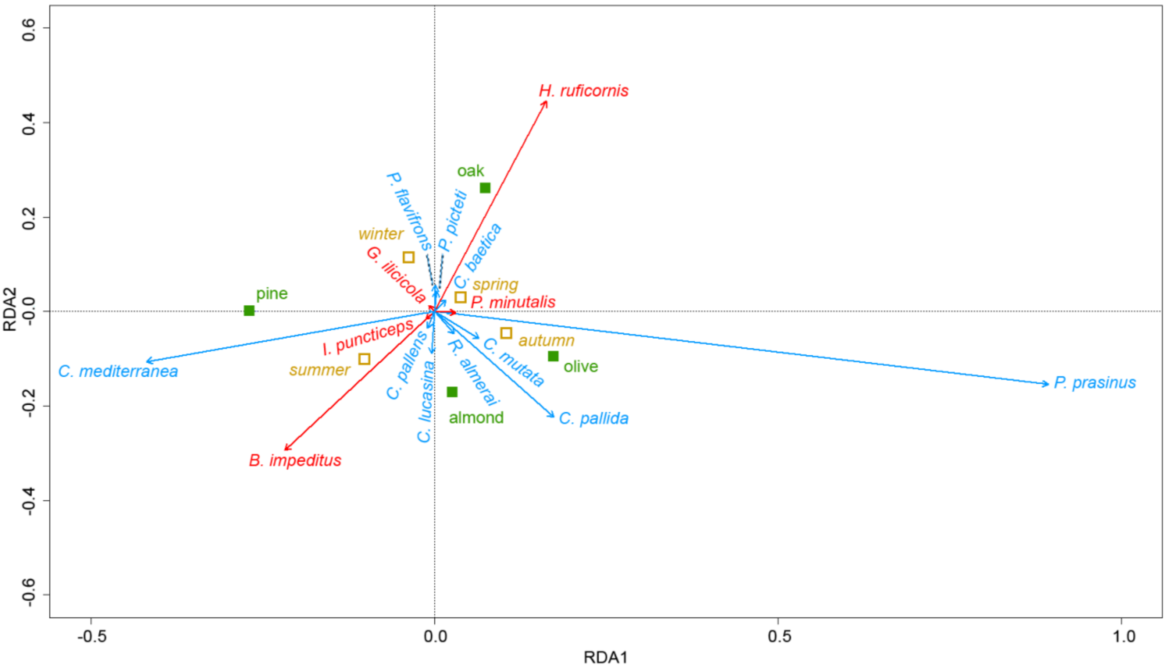

Figure 3. Redundancy analysis (RDA) triplot ordination showing variations in the abundance of the parasitoid complex (in red) and chrysopid species community (in blue) with respect to two nominal variables; tree species (in green) and season (in yellow).

5.5. Discussion

This study provides an insight into the abundance of chrysopid populations in olive groves, as well as almond, oak and pine trees adjacent to the crop, in addition to population decline factors. Juvenile stages of chrysopids were more abundant in almond, oak and olive trees than in pine trees. We found that parasitoids and chrysopids shared a similar temporal pattern in our study area. Additionally, the period of parasitoid incidence was found to extend beyond the April to November period previously reported (Alrouechdi 1984, Campos 1986). We observed that parasitoid abundance was highest in the summer months in olive trees, which is in line with the findings of Neuenschwander and Michelakis (1980) and Campos (1986).

The presence of juvenile stages (predated chrysopids and unknown factors) had a marked seasonal character, with the largest number in both categories recorded in summer, when the environment is less humid and temperatures are higher than in other seasons. This concurs with the results of previous studies which demonstrate that conditions, such as low humidity and high temperatures lead to increased mortality and slower development in the preimaginal stages (Daane 2001, El-Taeif et al. 2008, Pappas et al. 2008). This slower development could also render the juvenile stages more vulnerable to predators.

Overall, we found that mortality caused by parasitism (26.5%) constitutes a major chrysopid population decline factor. Although this is very similar to the level (27.7%) determined by Campos (1986) in olive groves in southern Spain, it is quite low compared to the levels (80% and 54.9%, respectively) reported in olive groves by Alrouechdi et al. (1981) in France and Neuenschwander and Michelakis (1980) in Crete.

With regard to tree species, the parasitism rate per tree in olive trees was very low as compared to previous studies (Neuenschwander and Michelakis 1980, Alrouechdi et al. 1981, Alrouechdi 1984, Campos 1986) and considerably lower than that in the three arboreal species (almond, oak and pine) studied. This, together with predation and unknown factors, make olive trees the most important arboreal species with regard to the number of viable next-generation adult chrysopids.

The highest rate of parasitism recorded in almond, oak and pine trees could be due to their location in seminatural areas bordering the crop. The seminatural habitats and landscape bordering the crop are characterized by greater species richness and parasitoid diversity than other types of habitat such as crop and vegetation cover (Inclan et al. 2015). Few data are available on the seasonality of parasitism in these trees. However, we demonstrated that the parasitism rate in pine and almond trees is higher in the summer months, which is similar to the pattern found by Judd (1949) in pine trees. Oak trees showed a more-or-less constant rate of parasitism throughout the year, which is similar to the rate of close to 15% recorded in other studies (Pantaleoni 1996). Additionally, oak trees become a parasitoid bank in winter due to their high rate of parasitism. This could have a negative effect on the next chrysopid generation and enable parasitoids to move into olive groves in spring. However, low rates of parasitism in olive trees and high rates in oak trees in spring suggest that parasitoids remain in oak trees. As almond trees have a high rate of parasitism in summer and are a good reservoir of juvenile chrysopids, they could play an important role in increasing chrysopid populations in olive groves in the summer months, when *P. oleae* are especially harmful to olive trees.

The chrysopid community is composed of ten species in our biotope, with, as already noted in previous studies, *P. prasinus* and the *C. carnea* complex accounting for the majority of species (Campos and Ramos 1983, Monserrat and Marín 1994, Porcel et al. 2017). On the other hand, studies focusing on the parasitoid complex of chrysopids have reported that a relationship exists between chrysopid species and their associated parasitoids (Clancy 1946, Muma 1959, New 1982). The parasitoid complex is composed of five species: three primary parasitoids (*B. impeditus*, *H. ruficornis* and *I. puncticeps*), with the highest levels of abundance, and two primary parasitoids, which also act as hyperparasitoids (*G. ilicicola* and *P. minutalis*), with the lowest levels of abundance.

B. impeditus, the most abundant species, affected a large number of chrysopids, mainly juveniles of the species *C. mediterranea*, *C. lucasina* and *C. pallens*, which were collected in almond and pine trees. Our results regarding this parasitoid, which is characterized by gregarious behaviour and emerges from the host through various orifices, corroborate the findings of previous studies (Clancy 1946, Alrouechdi et al. 1981). Although the period of activity of *B. impeditus* was similar to that in olive groves in Crete and France, the number of parasitoids per host was larger in our study (Neuenschwander and Michelakis 1980, Alrouechdi and Panis 1981).

The second most important parasitoid was *H. ruficornis*, which is found in Palearctic, Nearctic and Afrotropical regions (Townes 1977, Van Achterberg 2006, Buffington and Copeland 2016). This species has been previously cited in the Iberian Peninsula (Algarra et al. 1996), specifically in olive groves (Alrouechdi 1984, Campos 1986). Our findings would appear to contradict those of New (1982), who has stated that *H. ruficornis* is in a minority among species in the chrysopid parasitoid complex in Europe due to competition from other parasitoids for hosts. In our study, the second most abundant parasitoid *H. ruficornis*, which competed with four parasitoid species, plays a similar role to that observed by New (1982). Although little is known about its biology, *H. ruficornis* can, in our view, be classified as a solitary parasitoid, as only one parasitoid exits the host cocoon. This behaviour resembles that of other species of the same genus and concurs with other studies which suggest that all species of the genus *Helorus* are biologically similar (Clancy 1946, Principi 1948, Townes 1977, New 1982, Alrouechdi et al. 1984). *H. ruficornis* has also been shown to parasitize species of the genera *Chrysoperla*, *Pseudomallada*, *Chrysopa*, and *Nineta* (Killington 1932, Clancy 1946, Principi 1948, New 1982). We observed that *H. ruficornis* parasitizes the juvenile stages of the genera *Pseudomallada* (*P. picteti*, *P. flavifrons* and *P. prasinus*) and *C. baetica* which have a preference for oak trees in the Iberian Peninsula (Marín 1994, Monserrat and Marín 1994).

Of the two species from the genus *Isodromus* that parasitize chrysopids (Alrouechdi et al. 1984), we collected *I. puncticeps*, which is in a minority in the parasitoid complex studied. Although this resembles the pattern observed in Greek olive groves (Neuenschwander and Michelakis 1980, New 1982, Campos 1986, Pantaleoni 1996), *I. puncticeps* plays an important role in French olive groves (Alrouechdi et al. 1980, Alrouechdi et al. 1981, Alrouechdi and Panis 1981). With the aid of RDA analysis, although we found a positive relationship between the abundance of *B. impeditus* and *I. puncticeps*, given the insufficient number of individuals of the latter, we were unable to shed any light on this relationship. Nevertheless, as previously described by Clancy (1946) and Campos (1986), we found *I. puncticeps* to be a gregarious parasitoid.

Data on the role of the chrysopid parasitoid *G. ilicicola* in almond, oak and olive trees have never previously been cited in Spain (Falcó, in prep.). Despite the small number of individuals collected, *G. ilicicola* is not associated with any specific tree species. In this respect, species of the genus *Gelis* have been reported to be solitary ectoparasitoids from *C. carnea s.l.* pupae or prepupae in French olive groves and, in some cases, act as hyperparasitoids of *C. carnea s.l.* larvae parasitized by *B. impeditus* (Alrouechdi et al. 1981, Alrouechdi and Panis 1981, Alrouechdi 1984).

Among the group of species composed of chrysopid hyperparasitoids, we identified the gregarious P. minutalis, whose incidence, with only five parasitized juveniles collected, was very low. While *P. minutalis* has been previously cited in Spain as a possible hyperparasitoid of the Order Lepidoptera (Askew et al. 2001), this is the first time it has been cited in relation to chrysopids (Darling, in prep.). Although the parasitic technique used by *P. minutalis* is unknown, other species of this genus have been found to be gregarious ectoparasitoids. They have been observed to lay their eggs in aphid colonies or on the egg pedicel of chrysopids and to adhere to the chrysopid larva until the latter spins a cocoon and the parasitoid then devours the host (Smith 1917, Principi 1940, 1948, Alrouechdi et al.

1984, Gerling and Bar 1985). The small number of *P. minutalis* hyperparasitoids reported in our study could be due to its modus operandi to parasitize the host, which resembles that of other species from the genus *Perilampidae*.

While the characteristics that enable chrysopids to protect against natural enemies include the use of exogenous trash by juveniles as a defensive shield against predation (Eisner and Eisner 2002), evidence with regard to parasitism is less clear (Smith 1922, Muma 1959, New 1969). In our study, the rate of parasitism was found to be higher in naked chrysopid species (*C. lucasina*, *C. mediterranea*, *C. mutata*, *C. pallida* and *C. pallens*) as compared to trash-bearing species (*C. baetica*, *P. flavifrons*, *P. picteti*, *P. prasinus* and *R. almerai*); however Muma (1959) found that the rate of parasitism is lower in naked chrysopids than in more abundant trash-bearing chrysopids. Therefore, depending on chrysopid assemblage and abundance, as well as the parasitoid complex associated with each geographical area, rates of parasitism will, in our view, be affected by whether juvenile chrysopids are trash-bearing or naked. However further research is required to cast light on this relationship.

5.6. Conclusions

We have demonstrated that chrysopid abundance in almond and oak tree species in the arboreal stratum adjacent to olive groves is comparable to that in olive trees. With regard to population dynamics, the combined effect of three decline factors (parasitism, predation and unknown factors) of chrysopid populations over the short term needs to be taken into account when habitat management is being considered to conserve these populations. Additionally, in the biotope studied, we found that ten chrysopid species use the arboreal stratum to develop their biological cycle, in which *P. prasinus* is the most abundant species. We also found that three out of the five species in the parasitoid complex of the family Chrysopidae are primary parasitoids, with *B. impeditus* showing a preference for *C. pallens*, *C. lucasina* and *C. mediterranea*; and *H. ruficornis* being associated with *C. baetica*, *P. flavifrons* and *P. picteti*, representing the majority of parasitoid species. A knowledge of chrysopid population decline factors in seminatural habitats could be crucial for an effective habitat management program aimed at conserving and expanding chrysopid populations to boost the presence of chrysopids and the natural pressure on pests and to contribute to olive grove sustainability.

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5.8. Supplementary materials

Table S1. Multiple comparisons of GLMM abundance of juvenile stages of chrysopids in relation to tree species, site and month sampled including estimate, standard error (SE) and *p* value. Significance codes: ****P* <0.001, ** *P* <0.01, * *P* <0.05.

Variable	Comparison	Estimate	SE	*p* value	
Tree species	oak - almond	-0.1767	0.2287	0.86647	
	olive - almond	0.2701	0.2288	0.63853	
	pine - almond	-0.9539	0.2512	< 0.001	***
	olive - oak	0.4467	0.2156	0.16183	
	pine - oak	-0.7772	0.2363	0.00552	**
	pine - olive	-1.2239	0.2310	< 0.001	***
Site	Norberto – Los Almendros	0.84625	0.23155	0.00229	**
	Píñar (right) – Los Almendros	0.05453	0.27889	0.99967	
	La Pedriza – Los Almendros	0.09863	0.28184	0.99673	
	Píñar (left) – Los Almendros	-0.84413	0.26956	0.01456	*
	Píñar (right) – Norberto	-0.79172	0.23892	0.00804	**
	La Pedriza – Norberto	-0.74763	0.24434	0.01856	*
	Píñar (left) – Norberto	-1.69039	0.25117	< 0.001	***
	La Pedriza – Píñar (right)	0.04410	0.26624	0.99983	
	Píñar (left) – Píñar (right)	-0.89866	0.28928	0.01587	*
	Píñar (left) – La Pedriza	-0.94276	0.29044	0.01012	*
Month sampled	July16 - June16	0.033	0.19921	1	
	August16 - June16	-0.04597	0.20137	1	
	September16 - June16	-0.6002	0.21017	0.1487	
	October16 - June16	-1.575	0.23557	<0.001	***
	November16 - June16	-2.06946	0.25898	<0.001	***
	December16 - June16	-2.83195	0.31197	<0.001	***
	January17 - June16	-2.84347	0.32068	<0.001	***
	February17 - June16	-2.12561	0.2646	<0.001	***
	March17 - June16	-1.64999	0.24037	<0.001	***
	April17 - June16	-1.96602	0.26099	<0.001	***
	May17 - June16	-1.15665	0.22479	<0.001	***
	August16 - July16	-0.07897	0.19722	1	
	September16 - July16	-0.6332	0.20585	0.0834	
	October16 - July16	-1.60801	0.23308	<0.001	***
	November16 - July16	-2.10246	0.25581	<0.001	***
	December16 - July16	-2.86495	0.30851	<0.001	***
	January17 - July16	-2.87647	0.31837	<0.001	***
	February17 - July16	-2.15861	0.26253	<0.001	***
	March17 - July16	-1.68299	0.23745	<0.001	***
	April17 - July16	-1.99902	0.25908	<0.001	***
	May17 - July16	-1.18965	0.22219	<0.001	***
	September16 - August16	-0.55423	0.20675	0.2254	
	October16 - August16	-1.52903	0.23467	<0.001	***
	November16 - August16	-2.02348	0.2578	<0.001	***
	December16 - August16	-2.78598	0.30987	<0.001	***
	January17 - August16	-2.7975	0.31968	<0.001	***
	February17 - August16	-2.07964	0.26363	<0.001	***
	March17 - August16	-1.60402	0.23885	<0.001	***
	April17 - August16	-1.92005	0.2609	<0.001	***
	May17 - August16	-1.11068	0.22364	<0.001	***
	October16 - September16	-0.97481	0.24036	<0.01	**
	November16 - September16	-1.46926	0.26279	<0.001	***
	December16 - September16	-2.23175	0.31469	<0.001	***
	January17 - September16	-2.24327	0.32403	<0.001	***
	February17 - September16	-1.52541	0.26859	<0.001	***
	March17 - September16	-1.04979	0.24456	<0.01	**
	April17 - September16	-1.36583	0.26546	<0.001	***
	May17 - September16	-0.55645	0.23184	0.3909	

Continued Table S1

Variable	Comparison	Estimate	SE	*p* value	
Month sampled	November16 - October16	-0.49445	0.28263	0.8363	
	December16 - October16	-1.25694	0.33191	<0.01	**
	January17 - October16	-1.26847	0.34036	<0.01	**
	February17 - October16	-0.55061	0.28825	0.7414	
	March17 - October16	-0.07499	0.2664	1	
	April17 - October16	-0.39102	0.28653	0.9676	
	May17 - October16	0.41835	0.25561	0.8892	
	December16 - November16	-0.76249	0.34762	0.5408	
	January17 - November16	-0.77402	0.3566	0.557	
	February17- November16	-0.05616	0.30775	1	
	March17- November16	0.41946	0.28748	0.9478	
	April17- November16	0.10343	0.30481	1	
	May17- November16	0.9128	0.27685	0.0429	*
	January17- December16	-0.01152	0.39627	1	
	February17- December16	0.70634	0.35289	0.6792	
	March17- December16	1.18196	0.33567	0.0197	*
	April17- December16	0.86593	0.3505	0.3438	
	May17- December16	1.6753	0.32662	<0.001	***
	February17- January17	0.71786	0.3612	0.6899	
	March17- January17	1.19348	0.34413	0.0252	*
	April17- January17	0.87745	0.35842	0.3598	
	May17- January17	1.68682	0.33512	<0.001	***
	March17- February17	0.47562	0.29242	0.893	
	April17- February17	0.15959	0.31026	1	
	May17- February17	0.96896	0.28231	0.0285	*
	April17- March17	-0.31603	0.29025	0.9947	
	May17- March17	0.49334	0.25982	0.7488	
	May17- April17	0.80937	0.27614	0.1226	

Table S2. Multiple comparisons of GLMM parasitism in relation to tree species, site and month sampled including estimate, standard error (SE) and *p* value. Significance codes: *** *P* <0.001, ** *P* <0.01, * *P* <0.05.

Variable	Comparison	Estimate	SE	*p* value	
Tree species	oak - almond	-0.2526	0.2587	0.761	
	olive - almond	-1.7424	0.3189	<0.001	***
	pine - almond	-0.2108	0.2827	0.878	
	olive - oak	-1.4897	0.3016	<0.001	***
	pine - oak	0.0418	0.2926	0.999	
	pine - olive	1.5315	0.3253	<0.001	***
Site	Norberto – Los Almendros	-0.71149	0.24846	0.0322	*
	Píñar (right) – Los Almendros	-0.22743	0.33585	0.9594	
	La Pedriza – Los Almendros	-0.65332	0.37438	0.3941	
	Píñar (left) – Los Almendros	-0.99089	0.42913	0.1351	
	Píñar (right) – Norberto	0.48406	0.29848	0.4709	
	La Pedriza – Norberto	0.05818	0.33984	0.9998	
	Píñar (left) – Norberto	-0.27939	0.41113	0.9589	
	La Pedriza – Píñar (right)	-0.42588	0.3692	0.7692	
	Píñar (left) – Píñar (right)	-0.76346	0.4485	0.4202	
	Píñar (left) – La Pedriza	-0.33757	0.47338	0.9512	
Month sampled	July16 - June16	1.49404	0.25556	<0.001	***
	August16 - June16	1.18344	0.26201	<0.001	***
	September16 - June16	1.10039	0.29752	<0.001	***
	October16 - June16	0.82897	0.42708	0.6854	
	November16 - June16	-0.12214	0.66692	1	
	December16 - June16	0.08032	0.8091	1	
	January17 - June16	2.0855	0.60708	0.0232	*
	February17 - June16	0.50792	0.56622	0.9988	
	March17 - June16	1.04374	0.41918	0.2975	
	April17 - June16	0.38706	0.56283	0.9999	
	May17 - June16	-0.20285	0.42731	1	
	August16 - July16	-0.3106	0.18579	0.8516	
	September16 - July16	-0.39365	0.236	0.8536	
	October16 - July16	-0.66508	0.38744	0.8279	
	November16 - July16	-1.61618	0.64051	0.2784	
	December16 - July16	-1.41372	0.78589	0.7799	
	January17 - July16	0.59146	0.58136	0.9962	
	February17 - July16	-0.98612	0.54068	0.7648	
	March17 - July16	-0.4503	0.37874	0.9859	
	April17 - July16	-1.10698	0.53901	0.6033	
	May17 - July16	-1.69689	0.39098	<0.001	***
	September16 - August16	-0.08306	0.23919	1	
	October16 - August16	-0.35448	0.39136	0.9987	
	November16 - August16	-1.30558	0.64298	0.6202	
	December16 - August16	-1.10312	0.78731	0.9516	
	January17 - August16	0.90206	0.58366	0.9071	
	February17 - August16	-0.67552	0.54107	0.9794	
	March17 - August16	-0.1397	0.38336	1	
	April17 - August16	-0.79639	0.54061	0.932	
	May17 - August16	-1.38629	0.393	0.0165	*
	October16 - September16	-0.27142	0.41138	0.9999	
	November16 - September16	-1.22252	0.65552	0.7376	
	December16 - September16	-1.02007	0.79952	0.9756	
	January17 - September16	0.98512	0.59615	0.8611	
	February17 - September16	-0.59247	0.557	0.9944	
	March17 - September16	-0.05665	0.40542	1	
	April17 - September16	-0.71333	0.55514	0.9743	
	May17 - September16	-1.30323	0.42058	0.0666	
	November16 - October16	-0.9511	0.72103	0.9687	
	December16 - October16	-0.74864	0.85499	0.999	
	January17 - October16	1.25654	0.66456	0.721	

Continued Table S2

Variable	Comparison	Estimate	SE	*p* value	
Month sampled	February17 - October16	-0.32105	0.62972	1	
	March17 - October16	0.21478	0.50389	1	
	April17 - October16	-0.44191	0.63352	0.9999	
	May17 - October16	-1.03181	0.52158	0.6584	
	December16 - November16	0.20246	0.99469	1	
	January17 - November16	2.20764	0.83912	0.2222	
	February17 - November16	0.63006	0.8147	0.9997	
	March17 - November16	1.16588	0.71993	0.8767	
	April17 - November16	0.50919	0.81172	1	
	May17 - November16	-0.08071	0.73	1	
	January17 - December16	2.00518	0.95658	0.5715	
	February17 - December16	0.4276	0.93399	1	
	March17 - December16	0.96342	0.85254	0.9908	
	April17 - December16	0.30674	0.93292	1	
	May17 - December16	-0.28317	0.86282	1	
	February17 - January17	-1.57758	0.76314	0.5935	
	March17 - January17	-1.04176	0.66276	0.897	
	April17 - January17	-1.69845	0.75913	0.4673	
	May17 - January17	-2.28835	0.67734	0.0276	*
	March17 - February17	0.53582	0.62987	0.9993	
	April17 - February17	-0.12086	0.73481	1	
	May17 - February17	-0.71077	0.64119	0.9921	
	April17 - March17	-0.65668	0.62701	0.9951	
	May17 - March17	-1.24659	0.51427	0.3389	
	May17 - April17	-0.5899	0.63654	0.9984	

Impact of olive groves and adjacent ecological infrastructure on the abundance and adult species richness of the family Chrysopidae (Neuroptera)

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6.1. Abstract

Habitat management is a conservation biological control technique which helps to reduce the use of inputs in olive groves and to improve sustainability. Recent studies of olive groves have point out that vegetation cover, which provides food resources, as well as reproduction and refuge sites, increases chrysopid populations and diversity. However, little is known about the effect of seminatural habitats (SNHs) (bordering/patch vegetation). In this context, our study aimed to determine the attraction of adult chrysopids to different species in the arboreal stratum of the SNHs adjacent to olive groves. We also aimed to identify the factors responsible for this attraction: the presence, abundance and species richness of certain trees and plants in crop cover and adjacent vegetation patches, as well as the abundance of food, such as the pest *Prays oleae* (Bernad, 1788), available to chrysopid brood. We vacuum sampled 75 almond, oak, olive and pine trees fortnightly between April and October of 2016. Each tree, chosen at random and evenly distributed among five organic olive groves selected according to their availability, was vacuumed for two minutes. Oak trees recorded the highest abundance of adult chrysopids, while olive trees had the highest abundance of chrysopid larvae.

A total of 17 chrysopid species belonging to six genera were captured, of which *Pseudomallada prasinus* was the most abundant during the period of the study and had a preference for oak trees for its refuge and/or food. On the other hand, *Chrysoperla mutata* and *Chrysoperla pallida* showed a preference for olive trees.

Keywords: *Chrysoperla carnea* complex, seminatural habitats, bordering areas, adjacent vegetation patches, vegetation cover, *Olea europaea, Pinus halepensis, Prunus dulcis*, *Quercus rotundifolia*

6.2. Introduction

The greatest challenge facing agriculture today is to reduce the use of inputs in order to improve the sustainability of agroecosystems. This would mean focusing on ecological principles, efficient input use and organic pest management, while ensuring profitable crop yields without harming the environment (Reddy 2017).

Conservation biological control (CBC) is a sustainable approach to controlling pests, which could help to cut back the use of pesticides as part of an integrated pest management (IPM) strategy (Begg et al. 2017), especially in perennial crops (Rieux et al. 1999). CBC mainly aims to conserve and increase natural enemy populations (Barbosa 1998), in which seminatural habitats (SNHs) are an important tool; these habitats provide natural enemies with essential resources, such as pollen, nectar, honeydew, alternative hosts and prey, as well as refuge and reproduction sites (Landis et al. 2000, Duelli and Obrist 2003, Bianchi et al. 2013). As SNHs tend to diminish with the expansion and intensification of agricultural practices in olive groves (Gúzman-Alvarez et al. 2009), it is important to understand their impact on natural enemies in order to maintain and conserve their populations in olive groves.

EU member states are implementing Common Agricultural Policy agroenvironmental schemes, whose objectives include the protection and creation of SNHs, which are defined as “any habitat inside or outside crop land containing non-crop plant communities” (Holland et al. 2017). The European agricultural landscape has a wide variety of SNHs, whose functions and characteristics (vegetative composition, structure, shape and area) determine their ability to maintain natural enemies and to contribute to biological control (Holland et al. 2016). In this context, it has become clear how the incorporation of SNHs can increase the populations and diversity of natural enemies (Holland and Fahrig 2000, Sorribas et al. 2016, Albertini et al. 2017, Hatt et al. 2017, Pfister et al. 2017). Nevertheless, the relative importance of SNHs for pest control can vary drastically according to crop type, pest, predator, habitat management and landscape structure. These all need to be taken into account when designing measures to improve biological control services through habitat restoration and management (Tscharntke et al. 2016). It is therefore crucial to increase our knowledge of diverse SNHs in order to maximise their effectiveness in IPM programs (Tscharntke et al. 2012, Holland et al. 2016, Holland et al. 2017).

Chrysopids, which can be found in virtually all agricultural ecosystems, are efficient biological control agents in the natural enemy communities of agroecosystems (New 1975, McEwen and Senior 1998, Duelli 2001, Nicoli Aldini 2002, Monserrat 2016). Their larval stage feeds on a wide variety of small, soft-bodied insects and mites, as well as lepidopteran eggs and small larvae (Ridgway and Murphy 1984). While the adult stage of most species has a palyno-glycophagous diet, it depends on the vegetal substrate to feed on nectar, pollen and honeydew (Villenave et al. 2006, Devetak and Klokocovnik 2016). In fact, previous studies have demonstrated a certain connection between the vegetal substrate and chrysopids, some of which have a preference for arboreal species and others for herbaceous species (Nielsen 1977, Aspöck et al. 1980, Monserrat and Marín 1994, Sziraki 1996, Monserrat and Marín 2001, Duelli et al. 2002, Villenave-Chasset and Denis 2013). In Mediterranean olive groves, the predominant genera are *Chrysoperla* and *Pseudomallada* (Szentkirályi 2001). In particular, the *Chrysoperla carnea sensu lato* (Stephens, 1836) complex of species, which are present in olive groves all year round, play a key role in the predation of the eggs and larvae of the olive moth, *P. oleae*, one of the principal pests (Ramos et al. 1984, Campos 1989, Szentkirályi 2001). Furthermore, due to its agricultural importance, *C. carnea s.l.* was declared insect of the year in 1999 (Dathe 1999). Although some species of the *C. carnea s.l.* complex are well defined with respect to morphological characteristics, habitats, courtship songs and molecular techniques, and enable us to better understand their biology and ethology in the context of the CBC (Cordero-Rivera and Galicia-Mendoza 2017), their taxonomy has not been fully resolved (Monserrat 2016).

Previous studies of SNHs have revealed how chrysopids use vegetation cover as reproduction and feeding sites (McEwen and Ruiz 1994, Villenave et al. 2005, Villenave et al. 2006, Franin et al. 2016), and that vegetation cover even has a positive effect on chrysopid abundance and diversity in olive groves (Porcel et al. 2017). Earlier studies found that the arboreal stratum of adjacent SNHs sometimes boost and other times diminish chrysopid abundance in a diverse range of crops (Mignon et al. 2003, Sorribas et al. 2016). González et al. (2008) found that chrysopid abundance and diversity in an olive grove adjacent to *Pinus halepensis* Mill. trees were lower than in one bordered by *Quercus rotundifolia* Lam. trees.

In this context, our study aims to evaluate the attraction of adult chrysopids to different species in the arboreal stratum in SNHs adjacent to olive groves, on the one hand, and to identify the factors responsible for this attraction. These factors include the presence, abundance and richness of species of tree and certain plants in crop cover and adjacent vegetation patches, as well as the abundance of food resources, such as the pest *P. oleae,* available to chrysopid brood.

In the SNHs of olive groves studied, the predominant arboreal species are *Q. rotundifolia*, *Prunus dulcis* (Mill.) D. A. Webb and *P. halepensis*, which are located on the border of these olive groves, in vegetation patches and individually, we hypothesized that:

- the abundance of chrysopid captures in all arboreal species would differ,
- differences in chrysopid species richness in arboreal species would be observed and
- botanical species diversity in SNHs would affect the chrysopid community over time and the olive groves selected.

6.3. Material and Methods

6.3.1. Area of study

The study was carried out in the Montes Orientales region of Andalusia, situated 20 km in the north of Granada province, with an area of 49,926.9 hectares (ha) devoted to olive cultivation (Junta de Andalucía 2014). Interspersed in a landscape dominated by olive plantations bordering and/or inside the crops are vegetation patches and/or individual *P. halepensis*, *Q. rotundifolia, P. dulcis and Quercus coccifera* L. (Fagales: Fagaceae) trees and/or indigenous shrubs such as *Juniperus oxycedrus* L. (Pinales: Cupressaceae), *Cistus albidus* L. (Malvales: Cistaceae), *Cistus clusii* Dunal (Malvales: Cistaceae), *Genista cinerea* (Vill.) DC. (Fabales: Fabaceae), *Lavandula latifolia* Medik. (Lamiales: Lamiaceae), *Pistacia terebinthus* L. (Sapindales: Anacardiaceae), *Rosmarinus officinalis* L. (Lamiales: Lamiaceae), *Thymus mastichina* (L.) L. subsp. *mastichina* (Lamiales: Lamiaceae), *Thymus zygis* L. subsp. *gracilis* (Boiss) R. Morales (Lamiales: Lamiaceae) and *Ulex parviflorus* Pourr. (Fabales: Fabaceae). These types of vegetation are the remains of pre-crop vegetal formations following the ploughing and clearing of natural vegetation, increasing intensification of olive grove soil management, Montes Orientales reforestation and, in some cases, the establishment of plantations by farmers.

The weather conditions in the study area are typical for an oceanic bioclimate with seasonal rainfall, an upper meso-Mediterranean thermotype, a low subhumid ombrotype with an annual average temperature and precipitation of 14^{o}C and 672.3 mm, respectively (Valle Tendero et al. 2005).

For the study, we selected five organic olive groves (Table 1) located at similar altitudes of between 800 and 1,100 meters above sea level, covering an area of between 0.9 and 215 ha. All five olive groves have spontaneous vegetation cover, which is removed by mechanical mowing and/or grazing between April and May. In addition, during the post-harvest period, the soil was fertilized with organic matter, and crushed pruning waste was placed between the crop rows to create inert vegetation cover. The incidence of pests, such as *P. oleae* and *Bactrocera oleae* (Gmel.), and diseases, such as *Fusicladium oleagineum,* was remedied by timely and targeted treatment (one for pests and two for diseases) with products listed in Annex II of Commission Regulation (EC) no. 889/2008.

Table 1. Characteristics and availability of each tree species and number of tree species sampled in each sampling site per month sampled.

Site	Code	Coordenates	Area (ha)	Number of trees sampled				
				Almond	Oak	Olive	Pine	Total
Norberto	N	37°19'5.96"N; 3°34'9.92"O	4.3	25	15	15	25	80
La Pedriza	P	37°20'17.44"N; 3°33'39.21"O	0.9	-	15	15	25	55
Los Almendros	LA	37°22'24.76"N; 3°37'46.03"O	215	25-26	15	15	-	55-56
Píñar (right)	PR	37°24'14.29"N; 3°29'14.13"O	58	-	15	15	25	55
Píñar (left)	PL	37°24'40.93"N; 3°28'52.41"O	124	24-25	15	15	-	54-55
Total				**75**	**75**	**75**	**75**	**300**

6.3.2. Collection of Chrysopidae family specimens

Chrysopidae adults and larvae were captured in 300 trees per sampling, 75 trees per species – *Olea europaea* L. (olive), *Q. rotundifolia* (oak), *P. dulcis* (almond) and *P. halepensis* (pine) – randomly selected, with a minimum distance of 15 meters between trees to ensure spatial independence, whose distribution depended on their availability in the study area (Table 1). The tree canopy was vacuumed for two minutes up to a height of three meters with the aid of a pre-tested entomological aspirator (InsectaZooka; BioQuip®, Rancho Dominguez, CA, USA) for capturing small arthropods, especially chrysopids (Wilson et al. 1993, Macleod et al. 1994, Samu and Sarospataki 1995, Hossain et al. 1999, Doxon et al. 2011, Sanders and Entling 2011, Sorribas et al. 2016). This precise vacuum sampling technique enables arthropod fauna to be collected, specifically in individual trees and/or vegetation cover of interest, differs from other methods, such as water traps and McPhail traps, and can also collect larger quantities of arthropods than other methods such as pitfall and beating (Brook et al. 2008). A total of 13 samplings were carried out fortnightly between April and October 2016, a period marked by higher levels of arthropod abundance in olive groves (Ruano et al. 2004, Santos et al. 2007). The samples were cold-stored in the field and, in the laboratory, were then cleaned and classified before being stored at -20°C. In the laboratory, the chrysopids were separated from other arthropods and vegetal material, labelled and conserved at -20°C until catalogued; the remaining arthropods were conserved in 70% alcohol. The adult chrysopids captured were taxonomically identified under a stereomicroscope (Nikon SMZ 800; Nikon, Tokyo, Japan) up to specie level according the Monserrat key (2016).

6.3.3. Presence and incidence of P. oleae

The abundance of *P. oleae* adults was monitored using funnel traps (Econex TA027; Sanidad Agrícola Econex S.L., Santomera, Murcia, Spain) with a pheromone lure (Snailnex®; Sanidad Agrícola Econex S.L., Santomera, Murcia, Spain). Ten traps (two per site), placed at least 150 meters apart, were inspected monthly from April to November 2016, and every eight to ten days in the months of June and July. The number of adult specimens collected was expressed as the index of adults captured per trap per day (A.T.D) (Formula 1).

$$A.T.D = \frac{Number\ of\ adults\ captured\ in\ both\ traps}{Number\ of\ traps\ \times\ number\ of\ days\ elapsed} \tag{1}$$

To measure the incidence of each generation (phyllophagous, anthophagous and carpophagous) of *P. oleae* in the different olive groves, damage to specific vegetative organs in each generation was examined under a stereomicroscope.

Phyllophagous generation (April-May; two samplings) – 100 shoots were collected (10 shoots, selected at random, from 10 trees, selected at random, per site, per sampling), the shoots attacked were counted and the percentage (%) of shoots attacked per tree was calculated (Formula 2).

$$\%\ shoots\ attacked = \frac{Number\ of\ shoots\ attacked}{Number\ of\ shoots\ observed} \times 100 \tag{2}$$

Anthophagous generation (May-June; two samplings) – 100 inflorescences were inspected (two per shoot, selected at random, in each of the 50 shoots from 5 trees, selected at random, per site, per sampling); the incubated, hatched and predated eggs were counted in order to calculate the percentage (%) of inflorescences attacked (Formula 3) and predated eggs (Formula 4) per tree.

$$\%\ inflorescences\ attacked = \frac{Number\ of\ inflorescences\ attacked}{Number\ of\ inflorescences\ observed} \times 100 \tag{3}$$

$$\%\ eggs\ predated = \frac{Number\ of\ eggs\ predated}{Total\ number\ of\ eggs\ \ counted} \times 100 \tag{4}$$

Carpophagous generation (July; two samplings) – 100 fruits were examined (two randomly selected fruits per shoot in each of the 50 shoots from five trees, selected at random, per site, per sampling); the incubated, hatched and predated eggs were counted to calculate the percentage (%) of fruits attacked (Formula 5) and eggs predated (Formula 6) per tree.

$$\% \, fruits \; attacked \; = \frac{Number \; of \; fruits \; attacked}{Number \; of \; fruits \; observed} \times 100 \tag{5}$$

$$\% \, eggs \; predated = \frac{Number \; of \; eggs \; predated}{Total \; number \; of \; eggs \; counted} \times 100 \tag{6}$$

6.3.4. Sampling of flora and vegetation

The evaluation of the abundance and cover in certain vegetal formations, such as the herbaceous stratum, disproportionately depends on the observer (Greig-Smith 1983). In order to offset this problem, we used the non-destructive "*Point Quadrat*" method (Daget and Poissonet 1971). With the aid of a needle (two millimetres in diameter), this method records the presence or absence of herbaceous species in 100 contact points five centimetres apart in a 5 m long transect. A total of eight transects per site were used (four in the middle of the crop rows and four under the olive tree canopy) at intervals of least 50 m in a single sampling in May 2016.

In the other SNHs (bordering/patch vegetation), given the extent of the species to be sampled, we used the "*line intercept*" method (Canfield 1941) to record the interception range (in centimetres) of the vegetal species in a 25 m long transect, which was taken inside the vegetation patch and along the bordering habitat. A total of 60 transects (three per bordering/patch vegetation) 50 m apart were carried out in all sites in a single sampling in October 2017.

The vegetation indices to both methodologies ("*Point Quadrat*" and "*Line Intercept*") were calculated as follows:

- *Specific cover* (*S.Cov*$_{cover}$ to "*Point Quadrat*") (Formula 7) is expressed as the number of contacts to specie *i*, over total contacts (T.C.), which is 100. All these values were expressed as percentages (%) (Daget and Poissonet 1971).

$$S.Cov_{cover} = \frac{number \; of \; contact \; specie_i}{T.C.} \times 100 \tag{7}$$

- *Specific cover* (*S.Cov*$_{patch}$ to "*Line Intercept*") (Formula 8) is expressed as the interception range (in centimetres) by specie *i*, over the total transect length (T.L.) in centimetres, which is 2500. All these values were expressed as percentages (%) (Canfield 1941).

$$S.Cov_{patch} = \frac{interception \; range \; by \; specie_i}{T.L.} \times 100 \tag{8}$$

- *Dominance by family* is the specific cover at the family level to "*Point Quadrat*" and "*Line Intercept*" separately.
- *Diversity* was calculated on the basis of the Shannon index ($H'_{cover\ or\ patch}$) (Magurran 2004) (Formula 9), where P_i is the relative abundance for specie *i*, and S is the number of species found. To "*Point Quadrat*" P_i is the number of contacts for specie *i* over the sum of the number of contacts to all the species found (Formula 10). To "*Line Intercept*" P_i is the interception range (in centimetres) by specie *i* over the sum of the interception range (in centimetres) by all the species found (Formula 11).

$$H'_{cover\ or\ patch} = -\sum_{i=1}^{S} P_i \times \ lnP_i \qquad (9)$$

$$P_{i\ \text{"Point Quadrat"}} = \frac{number\ of\ contacts\ to\ specie_i}{\sum number\ of\ contacts\ to\ all\ species} \qquad (10)$$

$$P_{i\ \text{"Line Intercept"}} = \frac{interception\ range\ by\ specie_i}{\sum interception\ range\ by\ all\ species} \qquad (11)$$

Vegetal specimens, which were difficult to identify *in situ* (in both methodologies), were labelled and identified in the laboratory by Dr. Antonio García with the aid of the Flora Vascular de Andalucía Oriental key(2011).

6.3.5. Statistical analysis

All study data were entered and processed using two databases (entomological and botanical). The data were statistically processed with the aid of the R program version 3.5.0 (R Development Core Team 2017).

6.3.5.1. Abundance and taxonomic identification of chrysopid adults and larvae

Analysis began with an exploration of the total abundance of individuals (adults and larvae) collected in the four tree species according to recommendations made by Zuur et al. (2010). The sampling dates were grouped by month to simplify the study period and to improve their graphical representation.

We then analyzed the total abundance of chrysopid adults and larvae separately per tree using a generalized linear mixed model (GLMM), with a binomial negative distribution (Formulas 12 to 14) and a log link function for tree species and site as fixed factors and sampling date as the random factor (Formulas 15 and 16) with the aid of the "lme4" software package (Bates et al. 2014):

$$\text{Abundance of adults and larvae}_{ij} \sim \text{NB}(\mu_{ij}, k) \tag{12}$$

$$\text{E}(\text{Abundance of adults and larvae}_{ij}) = \mu_{ij} \tag{13}$$

$$\text{var}(\text{Abundance of adults and larvae}_{ij}) = \mu_{ij} + \frac{\mu_{ij}^2}{k} \tag{14}$$

$$\text{Log}(\mu_{ij}) = \text{Tree species}_{ij} + \text{site}_{ij} + \text{a}_j \tag{15}$$

$$\text{a}_j \sim \text{N}(0, \sigma^2_{\text{sampling date}}) \tag{16}$$

The models were selected according to Akaike information criteria (AIC) (Burnham and Anderson 2002), and their residuals were checked for uniformity using the “DHARMa” software package (Hartig 2018). The multiple comparisons in each model (adult and larvae abundance) for tree species and site variables were verified using Tukey’s post-hoc test with the “multcomp” software package (Hothorn et al. 2008). Redundancy analysis (RDA) was used to explore the possible relationship between the composition of chrysopid species (more than ten specimens captured) and environmental variables (tree species, site and month sampled). The results are shown with the aid of a correlation triplot with the “vegan” software package (Oksanen et al. 2018).

6.3.5.2.Incidence of P. oleae

The indices calculated (% shoots affected, % inflorescences attacked, % fruits attacked and % eggs predated) in each generation (phyllophagous, anthophagous and carpophagous) were analyzed separately to detect any significant differences between sites. First, the data were checked for normality using the Shapiro-Wilk test. After confirming their normality, the data were examined using Fisher’s least significant difference test (Fisher-LSD) with Bonferroni adjustment (Steel et al. 1997) and a confidence level of 95% (α=0.05). If the data were not found to be normal, the Kruskal-Wallis test, with Bonferroni adjustment, was used. In both cases, the “agricolae” software package was used (De Mendiburu 2014). Pearson correlations, with a confidence level of 95% (α=0.05), were also separately carried out between average adult and larvae abundance per site and the percentage (%) of predated eggs per site using R base functions.

6.3.5.3.Flora and vegetation

The diversity index according to the type of SNH sampled (cover and bordering/patch vegetation) was studied separately in order to detect differences between sites. We also investigated specific cover at family level in each site separately according to each type of SNH (cover and bordering/patch vegetation). Data were inspected normality using the Shapiro-Wilk test and, if found to be normal, were analyzed with the aid of the Fisher-DLS test with a Bonferroni adjustment (Steel et al. 1997) and a confidence level of 95% (α=0.05). On the other hand, if the data were not found to be normal, the Kruskal-Wallis test, with a Bonferroni adjustment, was used. The "agricolae" software package was used in both cases (De Mendiburu 2014). Canonical correspondence analysis (CCA) was used to verify the possible relationship between the composition of chrysopid species and the three most abundant families' common to the sites in cover and bordering/patch vegetation.

6.4. Results

A total of 3,477 chrysopids (2,918 adults as compared to 559 larvae; n=3,900 trees sampled) were captured between April and October of 2016. The smallest number of captures were in April (21 chrysopids; n=300 trees sampled), while the largest number of both adults and larvae were captured in September (800 chrysopids; n=600 trees sampled) (Table 2), with intermediate values being recorded in the other months.

Table 2. Abundance of chrysopid adults and larvae collected in almond, oak, olive and pine trees by month sampled.

Month	Tree species	Adults	Larvae	Total
April	Almond	1	1	2
	Oak	8	1	9
	Olive	4	4	8
	Pine	2	0	2
	Subtotal	15	6	21
May	Almond	67	3	70
	Oak	66	8	74
	Olive	65	13	78
	Pine	18	8	26
	Subtotal	216	32	248
June	Almond	166	24	190
	Oak	220	47	267
	Olive	75	61	136
	Pine	101	15	116
	Subtotal	562	147	709
July	Almond	47	38	85
	Oak	385	18	403
	Olive	60	65	125
	Pine	26	14	40
	Subtotal	518	135	653
August	Almond	48	37	85
	Oak	195	9	204
	Olive	102	29	131
	Pine	14	14	28
	Subtotal	359	89	448
September	Almond	102	19	121
	Oak	334	16	350
	Olive	263	43	306
	Pine	17	6	23
	Subtotal	716	84	800
October	Almond	55	7	62
	Oak	173	10	183
	Olive	279	44	323
	Pine	25	5	30
	Subtotal	532	66	598
Total		2,918	559	3,477

6.4.1. Abundance of adult chrysopids

The abundance of adults vacuum-sampled in the four tree species showed the following significant variations according to the GLMM model (Table 3 and 4): oak trees recorded by far the highest abundance of adult chrysopids per tree (1.42±0.07; 1,381 adults; n=975 trees sampled), followed by olive trees (0.87±0.05; 848 adults; n=975 trees sampled), almond trees (0.5±0.04; 486 adults; n=975 trees sampled) and pine trees (0.21±0.02; 203 adults; n=975 trees sampled).

With respect to the seasonal abundance of adult chrysopids associated with the different trees species, oak trees reached a peak in early July (2.73±0.32; n=75 trees sampled) and September (2.44±0.37; n=75 trees sampled) (Figure 1), while the abundance of chrysopids in olive trees increased steadily from the end of July until October and reached a peak first in late September (2.75±0.29; n=75 trees sampled) and then in October (2.24±0.24; n=75 trees sampled) (Figure 1). On the other hand, chrysopid abundance in both pine and almond trees peaked at the beginning of June (1.49±0.21 almond trees; n=75 trees sampled and 0.87±0.19 pine trees; n=75 trees sampled), while almond trees again reached a peak at the beginning of September (0.96±0.22; n=75 trees sampled) (Figure 1).

The abundance of adults collected by sites differed significantly (Tables 3 and 4). Norberto (0.92±0.05; 958 adults; n=1,040 trees sampled) recorded the highest levels of abundance as compared to the other sites: La Pedriza, Los Almendros, Píñar (right) and Píñar (left). Among the latter four sites, Píñar (left) (0.6±0.05; 424 adults; n=703 trees sampled) showed significantly lower abundance than Los Almendros (0.77±0.06; 557; n=727 trees sampled) (Table 4).

Table 3. Anova (type II Wald Chi square test) results of GLMM models (adult and larvae abundance), with Chi-squared value (χ^2), degrees of freedom (d.f.) and *p* value. Significance codes: *** *P* <0.001, ***P* <0.01, and * *P* <0.05.

Model	Variable	χ^2	d.f.	***p* value**	
Adult abundance	Tree species	541.34	3	<0.001	***
	Site	127.25	4	<0.001	***
Larvae abundance	Tree species	162.72	3	<0.001	***
	Site	95.64	4	<0.001	***

6.4.2. Abundance of chrysopid larvae

Although the sampling method was more oriented towards the capture of adult chrysopids, larvae, whose abundance in the tree species varied significantly in the GLMM model, were also collected (Tables 3 and 4): olive trees (0.27±0.02; 259 larvae; n=975 trees sampled), which stood our significantly from the other species, had the highest abundance of larvae, with pine trees (0.06±0.01; 62 larvae; n=975 trees sampled) showing an opposite trend, while almond and oak trees both recorded intermediate values without significant statistical differences.

The seasonal abundance of larvae associated with oak trees peaked at the end of June (0.49±0.08; n=75 trees sampled), while it increased steadily in olive trees from the beginning of May, reached a maximum in early July (0.67±0.12; n=75 trees sampled), diminished and did not increase again until the months of September and October (Figure 1). In almond trees, seasonal abundance increased from late May and peaked in early August (0.37±0.09; n=75 trees sampled), and, from then on, began to decline. On the other hand, pine trees reached peak abundance at the end of June (0.15±0.04; n=75 trees sampled) (Figure 1).

Distribution among sites (Tables 3 and 4) also varied significantly: Norberto (0.23±0.02; 235 larvae; n=1,040 trees sampled) presented the highest level of abundance compared to La Pedriza, Los Almendros, Píñar (right) and Píñar (left), which showed no significant differences in abundance.

Table 4. Multiple GLMM comparisons of chrysopid adult and larvae abundance by tree species and site, with estimate, standard error (SE) and *p* value. Significance codes: *** *P* <0.001, ***P* <0.01, and * *P* <0.05.

Model	Variable	Comparison	Estimate	SE	*p* value	
Adult abundance	Tree species	pine - olive	-1.66636	0.10525	<0.001	***
		almond - olive	-0.69839	0.08961	<0.001	***
		oak - olive	0.58252	0.07671	<0.001	***
		almond - pine	0.96797	0.11181	<0.001	***
		oak - pine	2.24888	0.10342	<0.001	***
		oak - almond	1.28091	0.08835	<0.001	***
	Site	Los Almendros – La Pedriza	0.02943	0.10334	0.9986	
		Norberto – La Pedriza	0.67359	0.09584	<0.001	***
		Píñar (left) – La Pedriza	-0.27907	0.10480	0.0592	
		Píñar (right) – La Pedriza	-0.09784	0.10323	0.8775	
		Norberto – Los Almendros	0.64416	0.08984	<0.001	***
		Píñar (left) – Los Almendros	-0.30850	0.09880	0.0152	*
		Píñar (right) – Los Almendros	-0.12727	0.10432	0.7386	
		Píñar (left) – Norberto	-0.95266	0.09451	<0.001	***
		Píñar (right) – Norberto	-0.77144	0.09714	<0.001	***
		Píñar (right) – Píñar (left)	0.18122	0.10483	0.4149	

Continued Table 4.

Model	Variable	Comparison	Estimate	SE	*p* value	
Larvae abundance	Tree species	pine - olive	-1.59061	0.14354	<0.001	***
		almond - olive	-0.78945	0.11267	<0.001	***
		oak - olive	-0.86548	0.11359	<0.001	***
		almond - pine	0.80116	0.16366	<0.001	***
		oak - pine	0.72513	0.16082	<0.001	***
		oak - almond	-0.07603	0.13400	0.94	
	Site	Los Almendros – La Pedriza	0.05493	0.15639	0.9966	
		Norberto – La Pedriza	0.75818	0.13455	<0.001	***
		Píñar (left) – La Pedriza	-0.36830	0.17279	0.2015	
		Píñar (right) – La Pedriza	0.03822	0.15883	0.9992	
		Norberto – Los Almendros	0.70325	0.11948	<0.001	***
		Píñar (left) – Los Almendros	-0.42322	0.15792	0.0551	
		Píñar (right) – Los Almendros	-0.01671	0.15485	1.0000	
		Píñar (left) – Norberto	-1.12648	0.14068	<0.001	***
		Píñar (right) – Norberto	-0.71996	0.13275	<0.001	***
		Píñar (right) – Píñar (left)	0.40652	0.17139	0.1196	

6.4.3. Taxonomic identification of chrysopids

A total of 2,918 adult individuals belonging to 17 species from six different genera of the family Chrysopidae were captured: *Chrysopa* (4), *Chrysoperla* (4), *Cunctochrysa* (1), *Italochrysa* (1), *Pseudomallada* (6) and *Rexa* (1) (Table 5).

The most abundant species were *Pseudomallada prasinus* (0.22±0.01; 862 individuals; n=3,900 trees sampled), followed by *Chrysoperla mutata* (0.2±0.01; 774 individuals; n=3,900 trees sampled), *Chrysoperla pallida* (0.13±0.01; 520 individuals; n=3,900 trees sampled) and *Chrysoperla lucasina* (0.11±0.01; 424 individuals; n=3,900 trees sampled). The other species, which ranged from 1 to 81 captures, were much less abundant: *Pseudomallada picteti*, *Cunctochrysa baetica*, *Pseudomallada granadensis*, *Chrysoperla mediterranea*, *Pseudomallada flavifrons*, *Chrysopa viridana*, *Chrysopa formosa*, *Pseudomallada ibericus*, *Rexa almerai*, *Chrysopa pallens*, *Chrysopa dorsalis*, *Italochrysa italica* and *Pseudomallada subcubitalis* (Table 5).

Using RDA analysis, tree species, month and sampling site were found to account for 54% of chrysopid species ordination, with the first two axes accounting for 71% of the variability. An adjusted R^2 of 47% also suggests that other important variables were not covered by the model. The correlation triplot showed that *P. prasinus* was positively correlated with oak trees and the months of August and September, while *C. mutata* and *C. pallida* also correlated positively with olive trees and the month of October. Moreover, the latter two species were negatively correlated with the group of species associated with pine trees (*C. mediterranea*, *P. granadensis* y *P. picteti*) and the months of April and May. Finally, *C. lucasina* correlated with almond trees and the months of June and July (Figure 2).

Tabld 5. Abundance of chrysopid adults (mean±SE) captured in almond, oak, olive and pine trees by month sampled. Species abbreviations: PFL - *Pseudomallada flavifrons* (Brauer, 1851), PGR - *Pseudomallada granadensis* (Pictet, 1865), PIB - *Pseudomallada ibericus* (Navás, 1903), PPI - *Pseudomallada picteti* (McLachlan, 1880), PPR - *Pseudomallada prasinus* (Burmeister, 1839), PSU - *Pseudomallada subcubitalis* (Navás, 1901), CLU - *Chrysoperla lucasina* (Lacroix, 1912), CME - *Chrysoperla mediterranea* (Hölzel, 1972), CMU - *Chrysoperla mutata* (McLachlan, 1898), CPA - *Chrysoperla pallida* Henry et al., 2002, CDO - *Chrysopa dorsalis* Burmeister, 1839, CFO - *Chrysopa formosa* Brauer, 1851, CPAL - *Chrysopa pallens* (Rambur, 1838), CVI - *Chrysopa viridana* Schneider, 1845, CBA - *Cunctochrysa baetica* (Hölzel, 1972), ITA - *Italochrysa italica* (Rossi, 1790), RAL - *Rexa almerai* (Navás, 1919).

Month	**Tree species**	***Pseudomallada***						***Chrysoperla***				***Chrysopa***				***Cunctochrysa***	***Italochrysa***	***Rexa***
		PFL	**PGR**	**PIB**	**PPI**	**PPR**	**PSU**	**CLU**	**CME**	**CMU**	**CPA**	**CDO**	**CFO**	**CPAL**	**CVI**	**CBA**	**IIT**	**RAL**
April	Almond	0	0	0	0	0.01±0.01	0	0	0	0	0	0	0	0	0	0	0	0
	Oak	0	0	0	0.01±0.01	0.07±0.03	0	0.01±0.01	0	0	0.01±0.01	0	0	0	0	0	0	0
	Olive	0	0	0	0	0	0	0.01±0.01	0	0.03±0.02	0	0	0	0	0	0	0	0.01±0.01
	Pine	0	0	0.01±0.01	0	0.01±0.01	0	0	0	0	0	0	0	0	0	0	0	0
May	Almond	0	0.01±0.01	0	*	0.25±0.06	0	0.11±0.03	0	0	*	0	0.06±0.03	*	0	0	0	0
	Oak	*	0.03±0.01	0	0.03±0.01	0.31±0.09	0	0.04±0.02	0	0	0.01±0.01	0	0	0	0	0.03±0.02	0	0
	Olive	0	0	0	*	0.18±0.05	0	0.18±0.05	*	0.02±0.01	0.01±0.01	0	0	0	0	0.01±0.01	0	0.01±0.01
	Pine	0	0.04±0.02	*	0.03±0.01	0.02±0.01	0	0.03±0.01	0	0	0	0	0	0	0	0	0	0
June	Almond	*	*	0	0.03±0.01	0.07±0.03	0	0.78±0.12	0	0.05±0.02	0.11±0.03	0	0.03±0.01	0.01±0.01	*	*	0	0
	Oak	0.02±0.01	*	0	0.04±0.02	0.36±0.07	0	0.57±0.09	0	0.05±0.04	0.35±0.07	0	*	0	0.01±0.01	0.05±0.02	0	0
	Olive	0	0	0	0.01±0.01	0.05±0.02	0	0.3±0.05	0	0.01±0.01	0.11±0.03	0	0	0	0	*	0	0
	Pine	0	0.04±0.02	0.01±0.01	0.09±0.03	0.03±0.01	0	0.41±0.08	0.047±0.02	0	0.02±0.015	0	0.01±0.01	0	0	*	0	*
July	Almond	0	0	0	0	0.08±0.03	0	0.09±0.03	*	0.01±0.01	0.09±0.03	0	0	0	0.02±0.02	0.01±0.01	0	0
	Oak	0.01±0.01	*	0	0.03±0.01	1.2±0.12	*	0.16±0.03	0.013±0.009	0.35±0.06	0.62±0.09	0	0.01±0.01	0	0.05±0.02	0.1±0.03	*	0
	Olive	0	0	0	0	0.05±0.02	0	0.06±0.02	0	0.04±0.02	0.22±0.05	0	0	0	0.03±0.01	0	0	*
	Pine	*	0	0	0	*	0	0.05±0.02	0.1±0.03	0	*	0	0	0	*	0	0	0
August	Almond	0	0	0	0	0.21±0.05	0	0	*	0.05±0.03	0.04±0.02	0	0	0	*	0	0	0
	Oak	0	0.01±0.01	0	0.09±0.03	0.74±0.11	0	0	0	0.27±0.07	0.07±0.03	0	0	*	0.02±0.01	0.09±0.03	0	0
	Olive	0	0	0	0	0.27±0.05	0	0.01±0.01	0	0.25±0.05	0.11±0.03	0	0.03±0.02	0	*	0	0	0
	Pine	0	0	0	0	0.05±0.02	0	0	0.04±0.019	0	0	*	0	0	0	0	0	0
September	Almond	0	0	0	0	0.33±0.06	0	0	0	0.19±0.05	0.15±0.04	0	0	0	0	0.01±0.01	0	0
	Oak	0.07±0.02	0.03±0.02	0.01±0.01	0.11±0.04	0.82±0.09	0	0	0	0.75±0.14	0.33±0.06	0	0	0	0.02±0.01	0.08±0.02	0	0
	Olive	0	*	0	*	0.19±0.04	0	0	0	1.22±0.13	0.31±0.05	0	0	0	0	0.01±0.01	0	0
	Pine	0	0.05±0.02	*	0	0.02±0.01	0	0	0.02±0.011	0.01±0.01	0	0	0	0	0	0	0	0
October	Almond	*	0	0	*	0.02±0.01	0	*	0	0.22±0.06	0.107±0.03	0	0	0	0	0	0	0
	Oak	0.07±0.02	0.04±0.02	0	0.05±0.02	0.39±0.07	0	0	0	0.31±0.05	0.29±0.05	0	0	0	0	*	0	0
	Olive	0	*	0	0	0.04±0.02	0	0.01±0.01	0	1.32±0.12	0.47±0.07	0	0	0	0	*	0	0
	Pine	0.01±0.01	0.05±0.02	0.01±0.01	0.03±0.01	0.02±0.01	0	*	0.013±0.009	*	0.01±0.01	0	0	0	0	0	0	0
Mean per species		0.01±0.002	0.01±0.002	*	0.02±0.003	0.22±0.01	*	0.11±0.01	0.01±0.002	0.20±0.01	0.13±0.01	*	*	*	*	0.02±0.003	*	*
Individuals captured		32	51	9	81	862	1	424	38	774	520	1	23	4	27	65	1	5

* Mean±SE ≤ 0.007 ± 0.007.

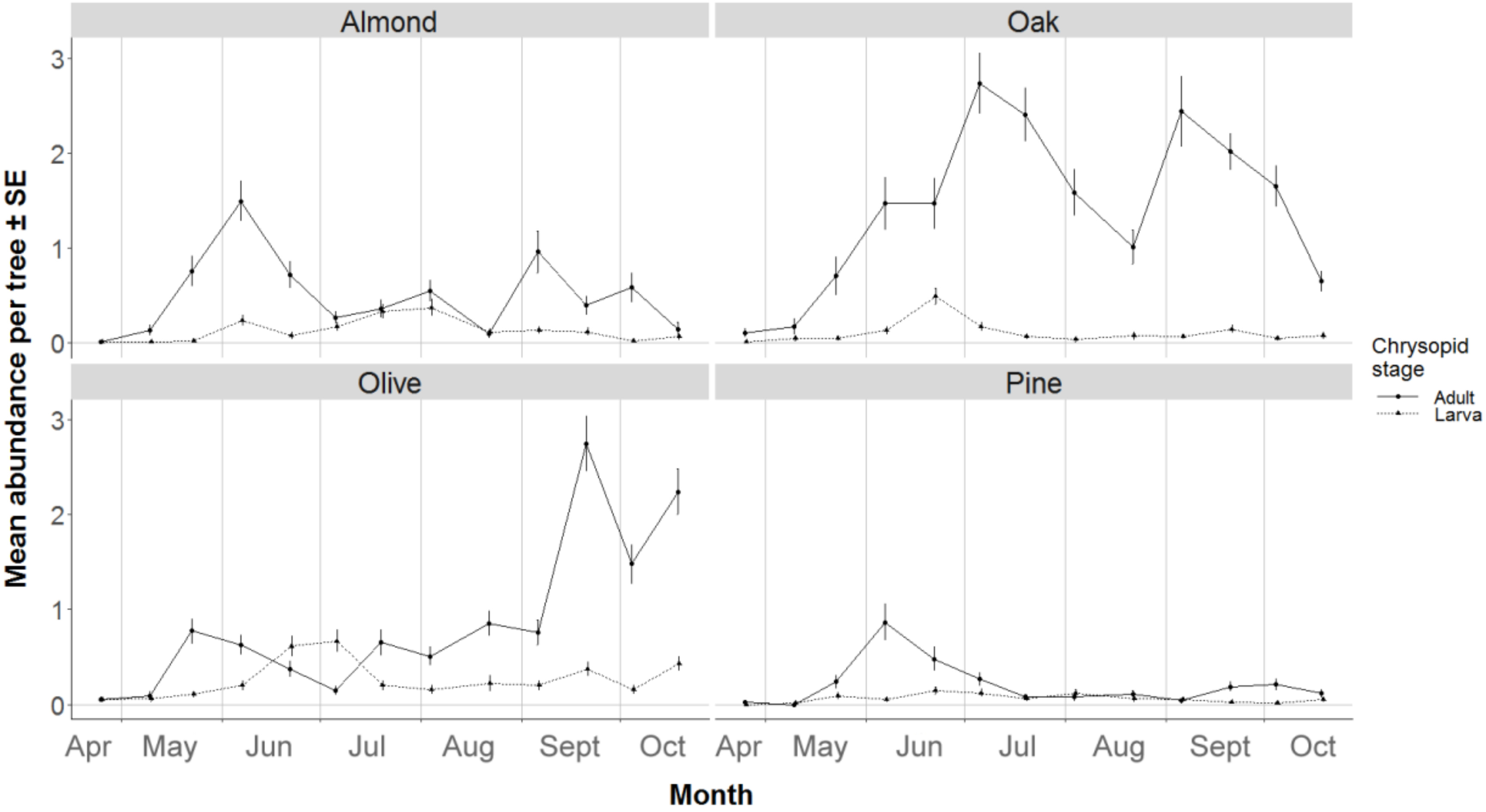

Figure 1. Seasonal evolution (mean±SE) of chrysopid adult and larvae in almond, oak, olive and pine trees by month.

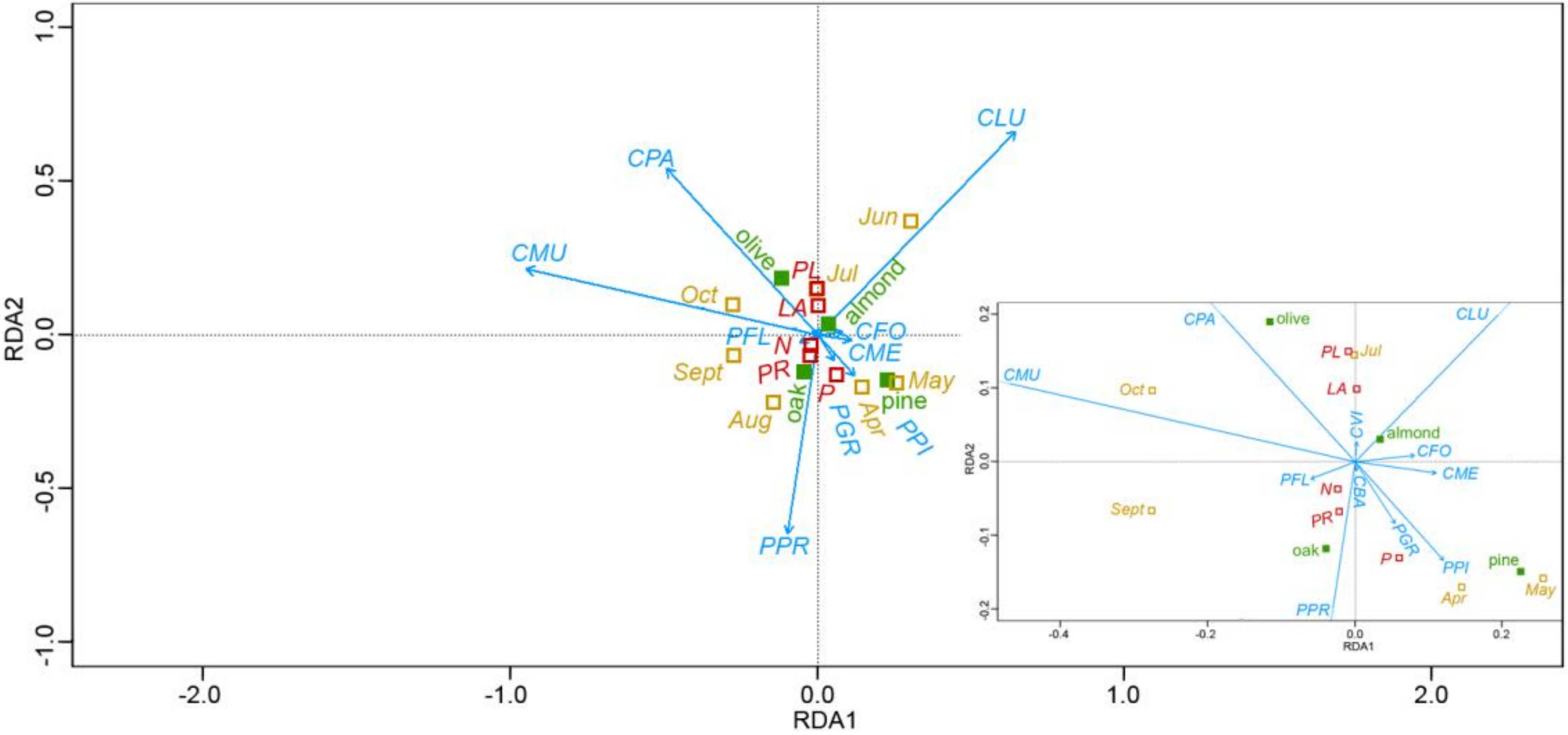

Figure 2. RDA triplot of chrysopid species by month sampled, site and tree species. For site code, see Table 1 and for chrysopid species code, see Table 5.

6.4.4. Presence and incidence of P. oleae

According to the ATD index, *P. olea* adults appeared in April, and continued to increase before reaching a peak at the end of June (217.4±36.3; n=10 funnel traps), when they began to decrease drastically before disappearing in August and then only reappeared in October (38.3±14.6; n=10 funnel traps).

With respect to the incidence of the phyllophagous generation of *P. oleae*, the percentage of shoots attacked was significantly higher in Norberto (90±2.3; n=200 shoots) than Los Almendros (77±2.9; n=200 shoots), although the incidence in these two sites did differ significantly from La Pedriza, Píñar (left) and Píñar (right) (Table 6).

With regard to the anthophagous generation, the average percentage of inflorescences attacked oscillated between 17.6 and 25.8% and did not differ significantly between sites (Table 6). On the other hand, differences were observed in the percentage of eggs predated, with La Pedriza (33±7.6; n=200 inflorescences) recording a higher percentage of predated eggs than Píñar (left) (5.5±1.7; n=200 inflorescences) and Píñar (right) (5.9±2.7; n=200 inflorescences) (Table 6). Furthermore, no differences were observed between the two Píñar sites or between Norberto, Los Almendros and La Pedriza (Table 6).

Analysis of the correlation between the percentage of predated *P. oleae* eggs and the abundance of chrysopid larvae (Pearson cor=-0.08, d.f.=4, *p*=0.902) and adults (Pearson cor=0.24, d.f.=4, p-value=0.695) in each site did not provide any significant results.

Finally, the average percentage of fruits attacked by the carpophagous generation of chrysopids ranged from 88.5 to 83.5 %, while no significant differences between sites were observed (Table 6). Nevertheless, the percentage of predated eggs in Norberto (87.1±2.2; n=200 fruits) and La Pedriza (84.2±2.1; n=200 fruits) sites was considerably higher than in Los Almendros, Píñar (left) and Píñar (right) (Table 6). Moreover, among the latter three sites, the percentage of predated eggs was significantly higher in Píñar (left) (70.5±3.4; n=200 fruits) than in Píñar (right) (55±4.1; n=200 fruits) (Table 6).

Analysis of the correlation between the percentage of predated eggs and the abundance of chrysopid larvae (Pearson cor=0.346, d.f.=4, *p*=0.569) and adults (Pearson cor=-0.05, d.f.=4, *p*=0.936) in each site did not produce any significant results.

Table 6. Analysis of variance in the incidence of *P. oleae* according to the indices calculated (mean±SE) in the phyllophagous (percentage of shoots attacked), anthophagous (percentage of inflorescences attacked and eggs predated) and carpophagous (percentage of fruits attacked and eggs predated) generations in La Pedriza, Los Almendros, Norberto, Píñar (right) and Píñar (left). Significant differences between olive groves are expressed as superindices marked with letters. The parameters Chisq, d.f. and *p* value are from the Kruskal-Wallis test and the parameter LSD – least significant difference – is from the Fisher-DLS test.

Generation	Index	Chisq	d.f.	*p* value	LSD	Site				
						La Pedriza	**Los Almendros**	**Norberto**	**Píñar (right)**	**Píñar (left)**
Phyllophagous	% shoots attacked	10.36	4	0.035		84.5±4^{ab}	77±2.9^{b}	90±2.3^{a}	87.5±3.1^{ab}	85.5±3.2^{ab}
Anthophagous	% inflorescences attacked				24.1	25.8±8.2^{a}	15.1±4.8^{a}	17.6±5.6^{a}	15.8±5^{a}	14.7±4.6^{a}
	% eggs predated	14.84	4	<0.01		33±7.6^{a}	13.2±5.2^{ab}	25.5±6.3^{ab}	5.9±2.7^{b}	5.5±1.7^{b}
Carpophagous	% fruits attacked	0.35	4	0.986		88.5±2.9^{a}	89.5±2.5^{a}	89.5±2.7^{a}	83.5±5.4^{a}	88.5±2.4^{a}
	% eggs predated	31.67	4	<0.001		84.2±2.1^{a}	69.3±2.9^{bc}	87.1±2.2^{a}	55±4.1^{c}	70.5±3.4^{b}

6.4.5. Flora and vegetation

6.4.5.1. Diversity and specific cover in vegetation cover

Diversity (Fisher-LSD d.f.=35, t value=2.99, LSD=0.52) in vegetation cover in Píñar (left) (1.03±0.09; n=8 transects) was significantly lower than in the other sites: Píñar (right), La Pedriza, Los Almendros and Norberto. Among the latter sites, La Pedriza showed significantly greater diversity than Píñar (right) (1.71±0.11; n=8 transects).

Although each site showed a particular specific cover at family level (Table 7), the families Asteraceae, Fabaceae and Poaceae were found to be the most abundant and relatively uniform in all sites.

Table 7. Results of variance analysis according to specific cover (mean±SE) at the family in vegetation cover in La Pedriza, Los Almendros, Norberto, Píñar (right) and Píñar (left). Significant differences between botanical families in each sampling site are expressed as superindices marked with letters.

Cover vegetation		**La Pedriza**	**Los Almendros**	**Norberto**	**Píñar (right)**	**Píñar (left)**
Statistical parameters	Chisq	34.67	29.16	22.4	36.57	25.07
	d.f.	10	11	9	7	6
	p value	<0.001	<0.001	<0.001	<0.001	<0.001
Botanical families	Apiaceae		1^{b}			
	Araceae		7.4 ± 2.1^{b}			
	Asteraceae	27.9 ± 4^{a}	75.8 ± 8.7^{a}	25.4 ± 6.9^{a}	51.6 ± 6^{a}	44.9 ± 5.1^{a}
	Brassicaceae	3.5 ± 2.5^{b}	16.4 ± 4.1^{ab}	5.7 ± 2.4^{ab}	2.2 ± 1^{c}	1.5 ± 0.5^{b}
	Cariophyllaceae	3.7 ± 0.8^{b}	3^{b}	3 ± 1.2^{ab}	6.8 ± 2^{bc}	3 ± 1.5^{b}
	Cistaceae	1^{b}				
	Convolvulaceae	3^{b}	2^{b}	1.5 ± 0.5^{b}		
	Fabaceae	16.9 ± 5.3^{ab}	16.9 ± 5.1^{ab}	21.2 ± 7.9^{ab}	52.5 ± 7.1^{a}	64.2 ± 6.5^{a}
	Geraniaceae	29.3 ± 6^{a}	1^{b}	11.4 ± 4.16^{ab}	17.9 ± 3.9^{b}	3 ± 1.2^{b}
	Malvaceae	5.8 ± 1.9^{b}	1^{b}	10.7 ± 5.6^{ab}		
	Papaveraceae				1^{c}	
	Plantaginaceae		25.8 ± 14.5^{b}			
	Poaceae	11.4 ± 2.5^{ab}	11.5 ± 3.2^{b}	17.7 ± 5.4^{ab}	10.6 ± 5.2^{bc}	4.4 ± 2.1^{b}
	Rubiaceae	4.2 ± 1.5^{b}	10 ± 4.9^{b}	2 ± 0.6^{b}	1.7 ± 0.7^{c}	2^{b}
	Veronicaceae	3 ± 1.5^{b}		1^{b}		

Of the 16 species in the family Asteraceae, the following species stood out: *Leontodon longirrostris*, *Crepis vesicaria* and *Anacyclus clavatus*. In the family Fabaceae (13 species), the most abundant species were: *Medicago minima*, *Medicago rigidula* and *Trifolium tomentosum*, while, in the family Poaceae (20 species), the most abundant species were: *Rostraria cristata*, Cynodon dactylon and *Hordeum murinum* subsp. *Leporinum.* A full list of species is given in Supplementary Material Table S1.

6.4.5.2. Diversity and specific cover in bordering/patch vegetation

The diversity (Kruskal-Wallis Chisq 15.03, d.f.=4, $p<0.01$) of bordering/patch vegetation in La Pedriza (1.6±0.1; n=9 transects) is significantly greater than that in Los Almendros, Píñar (left) and Píñar (right), but not in Norberto (1.1±0.2; n=6 transects). No differences were observed between the latter four sites.

The specific cover at the family level in each site was found to be highly variable. Certain families clearly dominated in some sites, while no families predominated in others (Table 8).

Table 8. Results of variance analysis according to specific cover (mean±SE) at the family level in bordering/patch vegetation in La Pedriza, Los Almendros, Norberto, Píñar (right) and Píñar (left). Significant differences between botanical families in each sampling site are expressed as superindices marked with letters.

Bordering/patch vegetation		**La Pedriza**	**Los Almendros**	**Norberto**	**Píñar (right)**	**Píñar (left)**
Statistical parameters	Chisq	29.03	50.8	19.44	35.6	11.04
	d.f.	10	13	12	8	5
	p value	<0.01	<0.001	0.08	<0.001	0.05
Botanical families	Anacardiaceae	2.6 ± 2.2^{b}	9.1 ± 5.3^{ab}			
	Apiaceae		2.6^{ab}			
	Asteraceae		0.6 ± 0.1^{b}	0.8		
	Boraginaceae				2.3 ± 0.3^{b}	
	Cariophyllaceae		0.4^{b}	4.2		
	Cistaceae	8.9 ± 3.3^{b}	1.9 ± 0.6^{b}	9.6±3.5	2.2 ± 0.9^{b}	7.1±4.3
	Cupressaceae	4.1 ± 1.5^{b}		12.6	11.2 ± 1.4^{ab}	
	Cyperaceae	0.6^{b}			0.8^{b}	
	Fabaceae	0.9 ± 0.5^{b}	15.9 ± 6.2^{ab}	5.4±3.4	4 ± 1.4^{b}	2.3±0.1
	Fagaceae	11.6 ± 2.4^{ab}	42.2 ± 6.2^{a}	7.6±3.6	41 ± 5.7^{a}	44.3±10
	Lamiaceae	5.5 ± 1.8^{b}	2 ± 0.8^{b}	4.9±1.7	7.3 ± 2.1^{b}	15.9±4.4
	Liliaceae		2.1 ± 1.2^{b}			
	Linaceae			0.6		
	Oleaceae	0.6^{b}		0.6		
	Pinaceae	33.9 ± 8.6^{a}		29.7±19.3	37.4 ± 3.6^{a}	
	Poaceae	9.6 ± 2.4^{b}	13.6 ± 4.2^{ab}	8.4±3.1	12.9 ± 3.6^{ab}	23.4±7.5
	Rhamnaceae	2.4^{b}	17.1 ± 7^{ab}			8.6
	Rosaceae		66.6 ± 19.2^{a}	55.7±4.6		
	Rubiaceae		0.4^{b}			
	Thymelaeaceae		1.2^{b}	2.5±1.1		

However, we identified the following five families in all the sites studied, with varying inter-site levels of abundance: Cistaceae, Fabaceae, Fagaceae, Lamiaceae and Poaceae. In addition, the families Rosaceae and Pinaceae (both planted) and Cupressaceae, though not found in all the sites, were observed to be highly abundant; for further information on vegetal species, see Table S2 in Supplementary Material.

6.4.5.3.Relationship between chrysopid species, flora and vegetation

Canonical correspondence analysis found no relationship between the most abundant chrysopid species and the most prominent botanical families in SNHs (cover and bordering/patch vegetation).

6.5. Discussion

The choice of the vacuum sampling method and the effort devoted to collecting the samples enabled us to obtain a representative sample of adult chrysopids and to record significant differences between the tree species and sites and to observe the seasonal movement of adult chrysopids in the period studied. The results obtained for olive grove chrysopids are in line with those published previously (Canard et al. 1979, Alrouechdi 1980, Campos and Ramos 1983, Porcel 2012).

Despite their uniform morphological structure, chrysopids have different biological and ecophysiological characteristics (Canard 1998). Thus, an increased knowledge of their seasonal adaptations at species level , especially about those that predominate in different geographical regions, is important in order to better understand the factors influencing their seasonal cycles and their ability to act against crop pests (Paulian 2001). In our study, oak trees were observed to receive the largest number of adult chrysopids, followed by olive and almond trees. In addition, seasonal variations in numbers of adults in each tree species were affected by the preference of the chrysopid community for different substrates. We captured 17 chrysopid species previously identified in olive trees in Spain (Campos and Ramos 1983, Monserrat and Marín 1994, González et al. 2008, Monserrat 2008, Porcel 2012, Monserrat et al. 2014, Monserrat 2016). With regard to the specificity of vegetal substrates, we observed that some species (*P. flavifrons*, *P. picteti*, *P. prasinus*, *C. formosa*, *C. pallens* y *C. baetica*) are ubiquitous and eurioic, with a preference for arboreal species, as previous studies have reported (Monserrat 2016). One special case in the ubiquitous and eurioic group is *C. lucasina,* known to prefer herbaceous vegetation, which was captured in considerable numbers in all four tree species up to the month of July. Other species collected (*P. ibericus* and *C. mediterranea*), which have a marked preference for conifers, were also captured in oak trees together with other chrysopid species (*C. viridana* and *C. baetica*). This was explained in previous studies by proximity of pine trees to oak trees (Monserrat 2016), given the proven migration and dispersion of chrysopids (Duelli 1984, 1986). On the other hand, two of the 17 chrysopid species, not known to have a substrate preference (Szentkirályi 2001, Monserrat 2016), one of which, *P. granadensis,* was more abundant in pine trees and the other, *C. mutata*, in olive trees, were identified, in all the tree species.

Four of the chrysopid species captured stand out in terms of their abundance: *P. prasinus*, *C. mutata*, *C. pallida* and *C. lucasina*

P. prasinus, which hibernates in the third larval stage (Canard 2005, Monserrat 2016), was the most abundant chrysopid species throughout the period of the study, which was also found to be the case in olive trees in south east France (Alrouechdi et al. 1980b, Alrouechdi et al. 1980a). Captures of *P. prasinus*, which has a marked preference for oak trees, began in April and ended in October, with maximum levels in July and September, which is in line with previous studies in Spain (Szentkirályi 2001). *P. prasinus* were also collected in almond and olive trees with a similar number of captures, while a negligible quantity was captured in pine trees. One explanation for this preference could be the form and density of an oak tree's canopy which may provide a better refuge for *P. prasinus* adults than almond, pine and olive trees.

The thermophile and xerophile specie*, C. mutata*, which hibernates as an imago, (Canard 2005, Monserrat 2016), appeared only in olive trees in April and May and later diversified to almond, olive and oak trees in June and July, before reaching a peak in oak trees in July. The capture curve of *C. mutata* indicates a preference for olive trees starting in August before reaching a maximum level in October. This behavior could be explained by its possible attraction to the vase shape of olive and almond trees when looking for food and open foliage, and as temperatures rise, *C. mutata* may look for cooler habitats, such as those provided by oak tree canopies. Finally, from August onwards, it returns to olive trees, possibly in search of warmer habitats and to feed on honeydew, which provide the elements needed to develop lipids to face the winter (Hagen and Tassan 1972, Lemesle et al. 1998).

C. pallida, whose behavior resembles that of *C. mutata*, appeared in olive trees in April. Despite diversifying to other tree species in later months, it showed a preference for oak trees and reached a peak in July when it began to decline until October. While decreasing in oak trees, *C. pallida* populations increased in olive trees and reached maximum abundance in October. Although its substrate preference is not known, *C. pallida* is largely associated with arboreal vegetation such as *Quercus*, *Olea*, *Pinus*, *Juniperus*, *Tamarix*, *Pistacia*, *Celtis* and *Rhamnus* (Monserrat 2016).

C. lucasina, an opportunist species that hibernates as an imago (Canard 2005, Monserrat 2016), is attracted to large patches of vegetation cover (Villenave et al. 2005). All the olive groves in our study contain vegetation cover at different degrees of development depending on mowing frequency and intensity. This vegetation cover can provide *C. lucasina* adults with food resources such as pollen and nectar containing essential saccharides in spring and summer (Villenave et al. 2005, Gonzalez et al. 2016). In our biotope, the tree species in SNHs adjacent to vegetation cover may be used by *C. lucasina* adults as a resting place, as previously pointed out by Duelli (2001). This could explain the captures of *C. lucasina* in almond, oak, pine and olive trees up to the month of July, where it may find refuge and/or food, especially in the summer months when vegetation cover is removed. Nevertheless, the floral architecture of cover species needs to be accessible to the body and mouthparts of *C. lucasina* to facilitate its consumption of these food resources (Van Rijn 2012, Nave et al. 2017). *C. lucasina,* which consumes only honeydew in autumn (Villenave et al. 2005), was captured in our study in olive trees in August and October. In our view this honeydew could come from *Euphyllura olivina* and *Saissetia oleae* which have tested positive in the laboratory (Villa et al. 2016).

Olive trees recorded a significantly higher abundance of *C. lucasina* larvae than other tree species and reached peak levels between late June and early July. This could be related to the attraction of chrysopids to *P. oleae*, whose carpophagous generation begins in this period time which also coincides with the highest rates of predation (Ramos et al. 1984). However, we did not find any correlation between the increase in chrysopids and the increase in the percentage of predated eggs in the different sites. This could be due to other factors, which influence the relationship between chrysopids and *P. oleae*, such as the temporal coincidence and distribution of *P. oleae* egg deposition (Campos 2001). Another possible explanation could be that the chrysopid community in our biotope and *C. carnea*, which are equally ubiquitous, lack a clear connection between egg deposition nearby and where Homoptera insects are present to provide their brood with food (Smith 1922, Neumark 1952).

On the other hand, chrysopid abundance at site level were very similar except for Norberto and Píñar (left), which had the highest and lowest levels of abundance, respectively, which shows that the chrysopid community in the area studied is quite stable. Given the presence of almond, oak, olive and pine trees in Norberto, we expect the broader diversity of habitat and food sources to attract a larger number of chrysopids, which was the case when vegetation cover was introduced into olive groves (Porcel et al. 2017) and when different SNHs (cover and hedgerow vegetation) were combined in organic clementine crops (Sorribas et al. 2016). Of the four tree species studied, we found that oak trees were the most attractive and pine trees the least attractive to chrysopids. This would appear to corroborate the results of González et al. (2008), who found that olive groves next to pine trees (*P. halepensis*) have lower levels of chrysopid abundance than those bordering oak trees (*Q. rotundifolia*).

Differences in the botanical diversity of vegetation cover observed in the different sites could be attributed to the various management system used. For example, in Píñar (right and left) olive groves, intensive seasonal sheep grazing could lead to the selection of certain species from the families Asteraceae and Fabaceae (García Fuentes et al. 2015).

We observed a lack of connection between the botanical families in SNHs (cover and bordering/patch vegetation) and the chrysopid community. This could be explained by the expansion of the olive groves and the landscape composition and the configuration of the SNHs (Fahrig 2013, Martin et al. 2019), as well as the ubiquitous, opportunist behaviour of chrysopids. In addition to feeding near their resting places, chrysopid adults consume pollen during part of the flowering period according to no precise pattern (Villenave et al. 2005, Villenave et al. 2006).

6.6. References

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6.7. Supplementary materials

Table S1. Specific cover “$S.Cov_{cover}$” (%) per specie (mean±SE), in vegetation cover in La Pedriza, Norberto, Los Almendros, Píñar (right) and Píñar (left).

Family	Species	La Pedriza	Norberto	Los Almendros	Píñar (right)	Píñar (left)
Apiaceae	*Torilis arvensis* (Huds.) Link			1		
Araceae	*Sonchus oleraceus* L.			7.38±2.07		
Asteraceae	*Anacyclus clavatus* (Desf.) Pers.		4.29±1.58	28±6.05		
Asteraceae	*Andryala ragusina* L.	2.5±1.5			1	
Asteraceae	*Anthemis arvensis* L.				2.5±0.5	5±2.81
Asteraceae	*Calendula arvensis* L.	5.75±3.47	2.5			2.67±1.2
Asteraceae	*Centaurea melitensis* L.				1	
Asteraceae	*Chamaemelum fuscatum* (Brot.) Vasc.	4.14±1.18				
Asteraceae	*Chondrilla juncea* L.	6.5±1.5	2	2		
Asteraceae	*Crepis vesicaria* L.	8.43±2.55	19±5.88	32.5±8.94	3	
Asteraceae	*Filago lutescens* Jord.		1		2	
Asteraceae	*Hedypnois rhagadioloides* (L.) F.W. Schmidt		2	9.38±3.36		
Asteraceae	*Hypochaeris glabra* L.					1
Asteraceae	*Leontodon longirrostris* (Finch & P. D. Sell) Talavera	9.25±2.04	7.67±2.67	6±3.27	49.13±6.4	40.63±4.83
Asteraceae	*Scorzonera laciniata* L.	2±0.45	1	1.2±0.2		
Asteraceae	*Senecio vulgaris* L.			3	1	
Asteraceae	*Taraxacum obovatum* (Willd.) DC.	8			3.5±0.5	
Asteraceae	*Urospermum picroides* (L.) F. W. Schmidt			1.5±0.5		
Brassicaceae	*Capsella bursa-pastoris* (L.) Medik.		3		2	1
Brassicaceae	*Diplotaxis catholica* (L.) DC.		4.67±2.33			2
Brassicaceae	*Diplotaxis erucoides* (L.) DC.			16.4±4.08		
Brassicaceae	*Diplotaxis viminea* (L.) DC.				2.2±1.2	
Brassicaceae	*Diplotaxis virgata* (Cav.) DC. subsp. *virgata*	3.5±2.5				
Cariophyllaceae	*Herniaria cinerea* DC.	1.25±0.25	1			
Cariophyllaceae	*Minuartia hybrida* (Vill.) Schischk. subsp. *hybrida*	2	1		9.33±3.71	1.33±0.33
Cariophyllaceae	*Petrorhagia nanteuilii* (Burnat) P. W. Ball & Heywood	1				
Cariophyllaceae	*Silene nocturna* L.	1				
Cariophyllaceae	*Silene tridentata* Desf.		1.5±0.5			
Cariophyllaceae	*Silene vulgaris* (Moench) Garcke	1	2			
Cariophyllaceae	*Stellaria pallida* (Dumort.) Piré	4±1.22	2±1		3.25±1.03	5

Continued Table S1

Family	**Species**	**La Pedriza**	**Norberto**	**Los Almendros**	**Piñar (right)**	**Piñar (left)**
Cistaceae	*Helianthemum salicifolium* (L.) Mill.	1				
Convolvulaceae	*Convolvulus arvensis* L.	3	1.5±0.5	2		
Fabaceae	*Astragalus hamosus* L.	2.5±0.5	3	5.57±2.09	1	2.5±0.5
Fabaceae	*Astragalus sesameus* L.	2.75±1.75	1.5±0.5			
Fabaceae	*Coronilla scorpioides* (L.) W. D. J. Koch			1		
Fabaceae	*Medicago littoralis* Loisel.	1.5±0.5				
Fabaceae	*Medicago minima* (L.) L.	6.2±3.37	8.25±5.31	1	27.63±4.69	62.88±6.4
Fabaceae	*Medicago orbicularis* (L.) Bartal.	7±3.21	1		1	
Fabaceae	*Medicago polymorpha* L.	3.6±1.6		14±7.57	2±0.7071	3
Fabaceae	*Medicago rigidula* (L.) All.	1	4.25±1.65	6.83±1.4	21.75±4.56	
Fabaceae	*Medicago truncatula* Gaertn.				5	
Fabaceae	*Trifolium scabrum* L.	7	6.2±3.32			
Fabaceae	*Trifolium tomentosum* L.	11.67±7.22	11±5.32			
Fabaceae	*Trigonella monspeliaca* L.	1	1.67±0.67	2.5±0.87	2.67±1.67	1.5±0.5
Fabaceae	*Vicia lutea* L. subsp. lutea				1	
Geraniaceae	*Erodium aethiopicum* (Lam.) Brumh. & Thell.	3.75±2.1			3.43±0.84	3±2
Geraniaceae	*Erodium chium* (L.) Willd.			1		
Geraniaceae	*Erodium malacoides* (L.) L´Hér. subsp. *malacoides*	22.4±8.98				
Geraniaceae	*Erodium primulaceum* (Lange) Lange		12.17±4.77			
Geraniaceae	*Geranium molle* L.	13.38±3.54	3.6±1.12		14.88±3.25	3
Malvaceae	*Malva cretica* Cav. subsp. *althaeoides* (Cav.) Dalby	5.8±1.85				
Malvaceae	*Malva neglecta Wallr.*			1		
Malvaceae	*Malva nicaensis* All.		10.67±5.55			
Papaveraceae	*Papaver rhoeas* L.				1	
Plantaginaceae	*Plantago lagopus* L.			3.33±2.33		
Plantaginaceae	*Plantago lanceolata* L.			29.75±16.71		
Poaceae	*Aira caryophyllea* L.		1			
Poaceae	*Avena barbata* Link			8.5±3.5	2	4.5±1.5
Poaceae	*Avena sterilis* L.	1.5±0.5		1.75±0.48		
Poaceae	*Bromus diandrus* Roth	1				
Poaceae	*Bromus hordeaceus* L.	3		5.67±4.18	1	
Poaceae	*Bromus rubens* L. subsp. *rubens*			4	4.5±1.32	3.67±1.52
Poaceae	*Bromus sterilis* L.	1			5.75±3.54	
Poaceae	*Catapodium rigidum* (L.) C. E. Hubb. subsp. *rigidum*	1.6±0.6	7.25±2.29			
Poaceae	*Cynodon dactylon* (L.) Pers.		1	14		
Poaceae	*Echinaria capitata* (L.) Desf.				2	

Continued Table S1

Family	**Species**	**La Pedriza**	**Norberto**	**Los Almendros**	**Píñar (right)**	**Píñar (left)**
Poaceae	*Hordeum murinum* L. subsp. *leporinum*	3.13±0.61	9.33±4.01	1.33±0.33	9.25±8.25	
Poaceae	*Lolium rigidum* Gaudin			4.17±1.14		
Poaceae	*Micropyrum tenellum* (L.) Link	1				
Poaceae	*Poa annua* L.		2			
Poaceae	*Poa bulbosa* L.	1				
Poaceae	*Rostraria cristata* (L.) Tzvelev	7.5±4.2	13±10			
Poaceae	*Vulpia ciliata* Dumort. subsp. *ciliata*		4.5±3.5		1	
Poaceae	*Vulpia myuros* (L.) C. C. Gmel.					
Poaceae	*Vulpia unilateralis* (L.) Stace	2.83±0.7				
Rubiaceae	*Callipeltis cucullaris* (L.) Steven	4	3			
Rubiaceae	*Galium aparine* L.		1		3	2
Rubiaceae	*Galium murale* (L.) All.	5			1	
Rubiaceae	*Galium parisiense* L. subsp. *parisiense*					
Rubiaceae	*Galium verticillatum* Lam.	2.67±0.88	2.5±1.5	10±4.93		
Veronicaceae	*Veronica agrestis* L.	3±1.53				
Veronicaceae	*Veronica polita* Fr.		1			

Table S2. Specific cover "*S.Cov*$_{patch}$" (%) per specie (mean±SE), in bordering/patch vegetation in La Pedriza, Norberto, Los Almendros, Píñar (right) and Píñar (left).

Family	Species	La Pedriza	Norberto	Los Almendros	Píñar (right)	Píñar (left)
Anacardiaceae	*Pistacia lentiscus* L.	0.04				
Anacardiaceae	*Pistacia terebinthus* L.	0.53		3.03±0.29		
Apiaceae	*Foeniculum vulgare* Mill.			0.14		
Asteraceae	*Helichrysum stoechas* (L.) Moench			0.07±0.01		
Asteraceae	*Staehelina dubia* L.		0.13	0.07±0.01		
Boraginaceae	*Lithodora fruticosa* (L.) Griseb.				0.38±0.03	
Cariophyllaceae	*Silene vulgaris* (Moench) Garcke		0.7	0.02		
Cistaceae	*Cistus albidus* L.	7.89±0.36	11.6±0.51			
Cistaceae	*Cistus clusii* Dunal		0.9±0.02		0.23	1.78±0.36
Cistaceae	*Fumana thymifolia* (L.) Webb		1.23±0.06	0.56±0.07		
Cistaceae	*Helianthemum cinereum* (Cav.) Pers. subsp. *rotundifolium*			0.4±0.02	0.7±0.13	
Cistaceae	*Helianthemum hirtum* (L.) Mill.		0.67			
Cupressaceae	*Juniperus oxycedrus* L.	3.2±0.17	2.1		1.87±0.12	
Cyperaceae	*Carex hallerana* Asso	0.07			0.07	
Fabaceae	*Argyrolobium zanonii* (Turra) P.W. Ball subsp. *zanonii*	0.04		0.02		
Fabaceae	*Astragalus incanus* L. subsp. *nummularioides*	0.16		0.12±0.03		
Fabaceae	*Genista cinerea* (Vill.) DC.		0.53	5.9±0.69	0.57	
Fabaceae	*Genista scorpius* (L.) DC.				0.18	0.38±0.01
Fabaceae	*Retama sphaerocarpa* (L.) Boiss.		0.233			
Fabaceae	*Ulex parviflorus* Pourr.		3.77±0.95	1.91±0.07	0.25	
Fagaceae	*Quercus coccifera* L.	5.56±0.27		3.76±0.26	4±0.68	0.87±0.07
Fagaceae	*Quercus faginea* Lam. subsp. *faginea*			0.91		
Fagaceae	*Quercus rotundifolia* Lam.	12.44±0.4	2.53±0.6	46.97±0.37	47.3±0.52	50.78±0.88
Lamiaceae	*Lavandula latifolia* Medik.			0.03	0.72	
Lamiaceae	*Phlomis lychnitis* L.		0.3		0.25±0.08	
Lamiaceae	*Rosmarinus officinalis* L.	6.31±0.28	4.4±0.47		7.03±0.26	11.88±0.49
Lamiaceae	*Teucrium pseudochamaepitys* L.	1.07±0.06	0.1	0.17±0.02	0.12±0.01	0.18
Lamiaceae	*Thymus mastichina* (L.) L. subsp. *mastichina*		0.83±0.08		0.05	
Lamiaceae	*Thymus zygis* L. subsp. *gracilis* (Boiss.) R. Morales	0.29	1.67±0.26	0.47±0.06	0.37±0.05	2.53±0.55
Liliaceae	*Asparagus acutifolius* L.			0.48±0.07		
Linaceae	*Linum suffruticosum* L.		0.1			

Continued Table S2

Family	**Species**	**La Pedriza**	**Norberto**	**Los Almendros**	**Piñar (right)**	**Piñar (left)**
Oleaceae	*Olea europaea* L.	0.07	0.1			
Pinaceae	*Pinus halepensis* Mill.	26.33±0.95	8.17		9.35±0.3	
Pinaceae	*Pinus pinaster* Aiton		1.73			
Poaceae	*Brachypodium retusum* (Pers.) P. Beauv. subsp. *retusum*	10.56±0.25	1.33	6.1±0.45	6.27±0.23	1.6±0.02
Poaceae	*Dactylis glomerata* L. subsp. *hispanica*	2.53±0.23	0.07	1.08±0.08	0.03	
Poaceae	*Macrochloa tenacissima* (L.) Kunth	6.16±1.05	5.13±0.13	1.5±0.72	9.35±0.68	8.15±0.74
Poaceae	*Piptatherum miliaceum* (L.) Coss.		0.5±0.18	0.41	0.45	
Rhamnaceae	*Rhamnus lycioides* L.	0.27		2.84±0.39		0.72
Rosaceae	*Prunus dulcis* (Mill.) D. A. Webb		27.87±0.76	11.1±1.07		
Rubiaceae	*Rubia peregrina* L.			0.02		
Thymelaeaceae	*Daphne gnidium* L.		0.83±0.18	0.07		

Attraction of green lacewings (Neuroptera: Chrysopidae) to native plants used as ground cover in woody Mediterranean agroecosystems

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Under rewiew in *Biological Control*

7.1. Graphical Abstract

		Biscutela auriculata	*Borago officinalis*	*Crepis capillaris*	*Echium plantagineum*	*Nigella damascena*	*Papaver rhoeas*	*Silene colorata*
Chrysopids in 2016/2017		✓/✓	✗/✓	✓/✓	✓/✓	✓/✓	✗/✓	✓/✓
Presence of adults/preimaginal		✓/✓	✓/✓	✓/✓	✓/✓	✓/✓	✓/✓	✓/✓
Type of prey	Acari	✗	✓	✓	✗	✓	✗	✓
	Aphid	✓	✓	✓	✓	✓	✓	✓
	Collembola	✓	✓	✓	✓	✓	✓	✓
	Thysanoptera	✓	✓	✓	✓	✓	✓	✓
No reported harmful effects on olive crop		✓	✓	✓	✗	✓	✓	✓
Good vegetative development		✓	✓	✓	✓	✓	✓	✓

7.2. Abstract

Using native seeds to establish semi-natural habitats is a novel strategy to restore biodiversity and ecosystem services such as biological control. Given that green lacewings (Neuroptera: Chrysopidae) are regarded as major biological control agents in different crops, the objective of this study was to test the attractiveness of selected native plant species to lacewings, and to measure plant effect on chrysopid abundance and/or species richness. During a two-year (2016-2017) field experiment near Villarrubia (Andalusia, Spain), 42 native plant species belonging to 13 families were planted in replicated plots. Plant development was monitored, and arthropods were vacuumed from each plot twice during the peak flowering period of May. Of the 36 plant species that developed well, green lacewings were observed to use 28 of these species to reproduce, feed and/or rest. The captured adults were mainly *Chrysoperla lucasina,* with some *Chrysoperla pallida.* No clear pattern of attraction common to all species was observed during the two-year sampling period. In 2017, eight plant species showed above-average chrysopid abundance and four of these were the most attractive to the green lacewings. In addition, Thysanoptera abundance correlated positively with chrysopid abundance. Given the attraction of chrysopids, the effect of pests and disease on olive orchards and satisfactory vegetative development, we consider *Biscutella auriculata*, *Borago officinalis*, *Silene colorata*, *Crepis capillaris, Nigella damascena* and *Papaver rhoeas* to be the native plant species best suited to host chrysopids and to restore ground cover in perennial Mediterranean crops.

Keywords. Non-crop vegetation; native seeds; ecological infrastructure; ground cover; chrysopids

7.3. Introduction

The general homogenisation of the agricultural landscape has led to a decrease in the insect population and diversity, resulting in a decline in ecosystem services such as biological pest control (Altieri and Nicholls, 2004; Penn et al., 2017). The European Union (EU) has been developing policy tools such as the agri-environment scheme (AES) (Science for Environment Policy, 2017) to "reduce environmental risks associated with modern farming on the one hand, and preserve nature and cultivated landscapes on the other hand" (European Commission, 2005), which will contribute to increasing crop sustainability (Tschumi et al., 2014).

Spanish legislation regulating pesticides includes the use of ecological infrastructure in the crops to protect and enhance natural enemy populations (RD 1311/14 September 2012). On-farm habitat of spontaneous or sown species is a good example of ecological infrastructure. Nevertheless the increased use of herbicides has led to a depauperate seed banks, and in order to create useful habitats for beneficial insects, it necessary to establish an ecological infrastructure through active sowing. One specific type of on-farm habitat for perennial woody crops (orchards) is the herbaceous understory between trees. There is growing interest and research into suitable plant species for ground cover restoration in woody Mediterranean agroecosystems such as olive, vineyards, citrus and almond orchards, as well as ecosystem services to prevent soil erosion and to maintain soil fertility (Alcántara et al., 2017; Gálvez et al., 2016). Additionally, the EU has been promoting programs such as The NAtive Seed Science, TEchnology and Conservation (NASSTEC) scheme on the use of native seeds to restore herbaceous communities in Europe. For agroecological applications, the seed mixtures that are currently available commercially are generally temperate forage species that are less suited to Mediterranean soil and weather conditions than to wild, native species (Hernández González et al., 2015). There is a great diversity of native plant species which are adapted to the climate conditions of orchards (Castroviejo, 1986-2012), and the use of native species is a novel strategy to ensure the success of ground cover restoration for crops. In addition, sowing these conservation seed mixtures could help to establish and maintain seed banks without the need for re-sowing and defend against invasion by non-native species and to reduce farming costs (Araj and Wratten, 2015; Siles et al., 2017). Additionally, regional native species contribute to increasing local arthropod biodiversity (Ruby et al., 2011) and provide cultural ecosystem services, such as wild food and medicinal plants, and to enhance landscape aesthetics (Nave et al., 2017).

The *Chrysoperla carnea s.l.* complex (Chrysopidae: Neuroptera), some of the most widespread and active predators, occupy an outstanding position among the wide variety of natural enemies of pests in agricultural areas (McEwen et al., 2001; Pappas et al., 2011; Porcel et al., 2017). In Europe, seven species belonging to this predator group have been characterized (Monserrat, 2016; Noh and Henry, 2010). *C. carnea s.l.* adults have a palyno-glycophagous diet of nectar and pollen resources (even insect honeydew) to obtain protein and carbohydrate, essential for their survival and reproduction (Canard et al., 1984; Gonzalez et al., 2016; Villa et al., 2016; Villenave et al., 2005). The larvae have a diet principally based on small arthropods such as aphids, thrips, mites, scales, springtails and moths, which may be supplemented with non-prey food resources, such as pollen and nectar, when prey are less abundant (Canard, 2001; Patt et al., 2003; Villa et al., 2016).

Ground cover vegetation contributes to supporting and increasing chrysopid populations, even on the canopy of perennial crops such as olives and apples. Thus, landscape management strategies to promote ground cover vegetation are expected to improve the biological control provided by chrysopids (Duelli, 2001; Porcel et al., 2017; Szentkirályi, 2001b; Wyss, 1995). However, the enhancement of natural enemies through landscape management does not necessarily lead to improved biological pest control due to numerous factors such as crop type, tritrophic interactions, intraguild predation, habitat, species synchrony, management regime and landscape. Because of this, it is necessary to identify the conditions that provide effective biological control (Bianchi et al., 2013; Rand et al., 2006; Tscharntke et al., 2016).

The complex trophic relationship between natural enemies and plants is influenced by various factors. The functional traits of flowers, as well as other factors such as the presence of prey, affect the attractiveness to natural enemies (Hatt et al., 2017; Nave et al., 2016; Van Rijn and Wackers, 2016). With respect to chrysopids, flowers with well-exposed nectaries are known to be a suitable food source (Van Rijn and Wackers, 2016), and the presence of available prey at the preimaginal stage could prompt the appearance of adults in plants containing these arthropods (Schultz, 1988). The fundamental aspects of this tri-trophic relationship are linked to reproduction and feeding. The habitats in which reproduction occurs can be identified by the presence of preimaginal stage arthropods characterized by limited mobility, while habitats which support adults are selected for feeding and/or resting purposes (Bianchi et al., 2013). Chrysopids, which use a wide range of habitats even in absence of prey, show less discriminatory oviposition (Duelli, 1984) than other predators such as syrphids and ladybirds which only lay eggs when prey abundance exceeds a certain threshold (Bianchi et al., 2013). With regard to feeding behaviour, given their generalist and opportunistic nature, *C. carnea s.l.* complex adults feed on a broad variety of plant species (Villenave et al., 2006).

It also needs to be taken into account that species belonging to the *C. carnea* and *Pseudomallada prasinus* complex could have different feeding behaviours due to their physiological and ecological differences (Denis and Villenave-Chasset, 2013; Henry and Wells, 2007). Thus, the range of *C. carnea* (Stephens, 1836) visitors is more diverse than that of *Chrysoperla lucasina* (Lacroix, 1912) visitors belonging to the complex mentioned above (Villenave et al., 2005). It is therefore essential to determine the specific requirements of each chrysopid species with respect to each plant species in order to select the appropriate plants that support natural enemies with their associated benefits (Chaplin-Kramer et al., 2013; Pantaleoni, 1996).

The objective of this study is to evaluate the attractiveness of selected native plant species to lacewings, their impact on chrysopid abundance and/or species richness and their correlation with potential prey abundance. We hypothesized that chrysopid abundance could be increased by the presence of particular plants in the area surveyed and/or depends on prey abundance. Both these hypotheses could be important factors in explaining the attraction of lacewings. In addition, based on previous studies by Denis and Villenave (2009) and Villenave et al. (2006), we expect to collect different *C. carnea s.l.* complex species in most of the plant species and in all the family plants sown given the low selectivity of their chrysopid oviposition behaviour.

7.4. Materials and Methods

7.4.1. *Site description and plant species*

The experiment was conducted in two growing seasons from November 2015 to June 2017 at an experimental farm in the village of Villarrubia (Andalusia, Spain; 37°49'49"N, 4°54'20"W). The field was bordered, to the north, by a commercial orange orchard and various irrigated crops and, to the south, by the River Guadalquivir with riverbank vegetation. Olive plantations are also located near the experimental farm. The soil is a Calcaric Fluvisol with a neutral/basic pH (IUSS Working Group, 2015). During the two-year study, meteorological conditions varied slightly, with a mean temperature in May 2017 3^{o}C higher than in May 2016 and rainfall in May 2017 half that in May 2016 (AEMET meteorological station, 37°50'56"N, 4°50'48"W).

Sown plant species were selected according to the following criteria (Frischie, 2017): (1) native angiosperms, (2) annual plants (ptherophytes), (3) plant height of less than 1 m (Castroviejo, 1986-2012), (4) flowering season in May before olive blooming (winter annuals), (5) self-seeding (Castroviejo, 1986-2012), (6) low water competition, especially against trees (7) and high erosion control potential.

The experimental area was 145x23 m in 2016 (Figure 1a) and 169x163 m in 2017 (Figure 1b). The area was tilled in late November of 2015 and 2016, and the seeds were planted in the days that followed. Weeds were managed by manual and mechanical methods three times per year and the plots were irrigated using overhead sprinklers once during germination and several times when required during plant development.

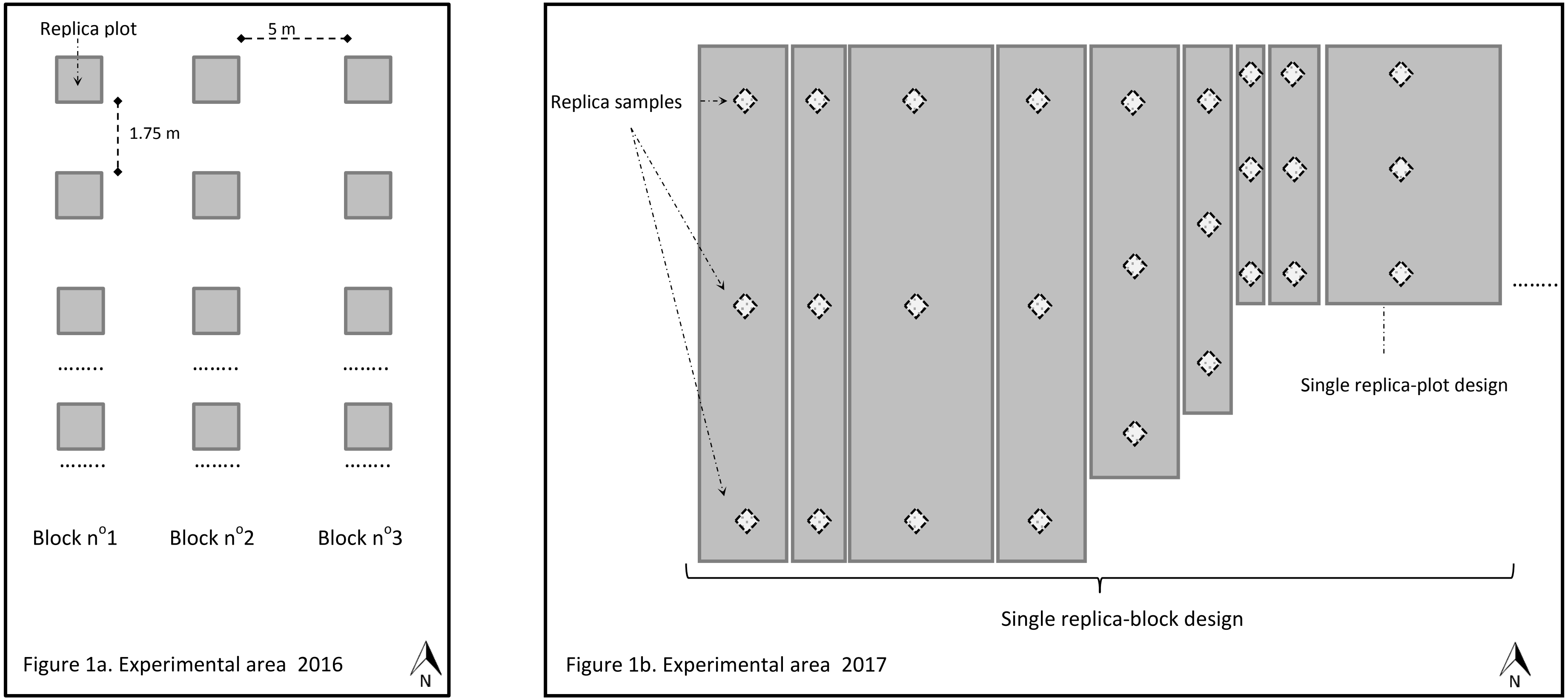

Figure 1. Diagrams showing planting and sampling design in 2016 (Fig. 1a): Three block with a replica plot 3x3 m, and 2017 (Fig. 1b): Single block with three replica plot 3x3 m.

7.4.2. *Arthropod collection*

In both years of the study, we sampled three 3x3 m squares for every plant sown at the beginning and end of May. However, the plants were sown differently each year: in 2016, a total of 40 plant species from 13 botanical families (Table 1) were planted in a completely randomized design in three blocks. Each plant species was sown in three 3x3 m squares (replica plots), with a 1.75 m interval between replica plots in the same block and a 5 m interval between replica plots in the three different blocks (total of 120 replica plots, Figure 1a).

Table 1. Plant species studied, surface sown, presence of chrysopids and mean plant cover (%) in 2016 and 2017. Y – Yes, N – No and NA – Not applicable.

Family	***Species***	***Plant code***	***Seed 2016 (m^2)***	***Seed 2017 (m^2)***	***Chrysopids collected (2016)***	***Chrysopids collected (2017)***	***Mean plant cover (%) (2016)***	***Mean plant cover (%) (2017)***
Apiaceae	*Tordylium maximum L.*	TOMA	Y (9)	N	Y		100	
Asteraceae	*Anthemis cotula L.*	ANCO	Y (9)	Y (1467)	N	Y	95	70
Asteraceae	*Calendula arvensis M.Bieb*	CAAR	Y (9)	Y (630)	N	N	100	100
Asteraceae	*Crepis capillaris (L.) Wall.*	CRCA	Y (9)	Y (50)	Y	Y	95	95
Asteraceae	*Glebionis segetum L.*	GLSE	Y (9)	Y (978)	Y	Y	100	98.3
Asteraceae	*Tolpis barbata (L.) Gaertn.*	TOBA	Y (9)	Y (50)	N	Y	33.3	60
Boraginaceae	*Borago officinalis L.*	BOOF	N	Y (489)		Y		80
Boraginaceae	*Echium plantagineum L.*	ECPL	Y (9)	Y (2632)	Y	Y	86.7	100
Brassicaceae	*Biscutella auriculata L.*	BIAU	Y (9)	Y (214)	Y	Y	100	100
Brassicaceae	*Capsella bursa-pastoris (L.) Medik.*	CABU	Y (9)	N	NA		83.3	
Brassicaceae	*Moricandia moricandioides Boiss.*	MOMO	Y (9)	Y (978)	Y	Y	100	95
Caryophyllaceae	*Silene colorata Poir.*	SICO	Y (9)	Y (232)	Y	Y	100	100
Caryophyllaceae	*Silene gallica L.*	SIGA	Y (9)	Y (489)	N	Y	95	80
Caryophyllaceae	*Vaccaria hispanica (Mill.) Rauschert*	VAHI	Y (9)	Y (489)	Y	NA	100	80
Cistaceae	*Helianthemum ledifolium (L.) Mill.*	HELE	Y (9)	N	NA		0	
Cistaceae	*Tuberaria guttata (L.) Fourr.*	TUGU	Y (9)	N	NA		0	
Dipsacaceae	*Scabiosa atropurpurea L.*	SCAT	Y (9)	Y (489)	Y	Y	95	
Fabaceae	*Anthyllis vulneralia L.*	ANVU	Y (9)	N	N		78.3	
Fabaceae	*Medicago orbicularis (L.) Bartal.*	MEOR	Y (9)	Y (163)	Y	NA	100	0
Fabaceae	*Medicago polymorpha L.*	MEPO	Y (9)	Y (163)	N	NA	100	0
Fabaceae	*Trifolium angustifolium L.*	TRAN	Y (9)	Y (1560)	Y	Y	33.3	100
Fabaceae	*Trifolium hirtum All.*	TRHI	Y (9)	Y (163)	N	Y	81.7	80
Fabaceae	*Trifolium lappaceum L.*	TRLA	Y (9)	Y (163)	N	Y	50	70
Fabaceae	*Trifolium stellatum L.*	TRST	Y (9)	N	Y		90	
Lamiaceae	*Cleonia lusitanica L.*	CLLU	Y (9)	N	Y		86.7	
Lamiaceae	*Salvia verbenaca L.*	SAVE	Y (9)	N	N		75	
Lamiaceae	*Stachys arvensis L.*	STAR	Y (9)	Y (100)	N	Y	45	90
Papaveraceae	*Papaver dubium L.*	PADU	Y (9)	Y (489)	N	Y	58.3	100
Papaveraceae	*Papaver rhoeas L.*	PARH	N	Y (5640)		Y		100

Continued Table 1

Family	***Species***	***Plant code***	***Seed 2016 (m^2)***	***Seed 2017 (m^2)***	***Chrysopids collected (2016)***	***Chrysopids collected (2017)***	***Mean plant cover (%) (2016)***	***Mean plant cover (%) (2017)***
Poaceae	*Aegilops geniculata Roth.*	AEGE	Y (9)	N	NA		0	
Poaceae	*Aegilops triuncialis L.*	AETR	Y (9)	N	NA		0	
Poaceae	*Anisantha madritensis L.*	ANMA	Y (9)	N	N		100	
Poaceae	*Anisantha rubens L.*	ANRU	Y (9)	N	N		100	
Poaceae	*Bromus hordeaceus L.*	BRHO	Y (9)	N	Y		100	
Poaceae	*Bromus scoparius L.*	BRSC	Y (9)	N	N		100	
Poaceae	*Cynosurus echinatus L.*	CYEC	Y (9)	N	Y		91.7	
Poaceae	*Hordeum murinum, subsp. leporinum L.*	HOMU	Y (9)	N	Y		95	
Poaceae	*Lolium multiflorum Lam.*	LOMU	Y (9)	N	Y		100	
Poaceae	*Trachynia distachya (L.) Beauv.*	TRDI	Y (9)	N	Y		100	
Ranunculaceae	*Nigella damascena L.*	NIDA	Y (9)	Y (978)	Y	Y	90	80
Scrophulariaceae	*Misopates orontium (L.) Raf.*	MIOR	Y (9)	N	N		41.7	
Veronicaceae	*Anarrhinum bellidifolium (L.) Willd.*	ANBE	Y (9)	N	NA		0	

In 2017, based on the chrysopid abundance results for 2016, 22 plant species from nine botanical families were planted, 20 of which had been planted in 2016 and two (*Borago officinalis* and *Papaver rhoeas)* were only planted in 2017. A single randomized replica-block and replica-plot design was used for each plant species, and the replica-plot design area ranged from 50 m^2 to 5,640 m^2. We collected three samples (replica samples) located equidistantly from the centre of each replica plot to avoid a border effect (Figure 1b).

The phenological stage and index of plant cover were recorded throughout the growing season for each plot.

After six months, when the plant species had reached optimal flowering, the arthropods were sampled by suction sampling of well-developed plant species throughout the covered surface: 34 plant species in 2016 and 19 in 2017 (Table 1).

Replica plots and replica samples for each plant species were vacuumed for 40 seconds using an insect aspirator (InsectaZooka, BioQuip® Products Inc., Rancho Dominguez, CA, USA). We then stored the samples at -20^{o}C, which were later sorted in the laboratory. Arthropods (chrysopids and potential prey) were identified to the highest taxonomic level required to determine their functional group. To assess the function of each plant species at species level, we distinguished between the different development stages of the chrysopids found in each plant species: preimaginal (eggs and larvae) and adults (males and females) stages. Chrysopid adults were determined up to species level as described according to the latest survey of the Iberian chrysopids (Monserrat, 2016). It is important to note that we carried out our study in both years on plants under similar conditions (phenology, location and sampling time).

7.4.3.*Statistical analysis*

Data analysis was carried out according to the protocol described by Zuur et al. (2010). Total chrysopid abundance per plant species was expressed as the sum of adult and preimaginal stages in each replica plot and replica sample, while total potential prey per plant species was expressed as the sum of Acari, Aphididae, Collembola and Thysanoptera in each replica plot and replica sample.

Differences in mean chrysopid abundance per year were tested using the Kruskal-Wallis test according to the Bonferroni correction method, as the data do not follow a normal distribution.

Total chrysopid abundance per year was separately modelled by fitting a generalized linear model (GLM) with a negative binomial distribution as a function of plant species and potential prey abundance. We used only plant species in which chrysopids were recorded (19 in 2016 and 18 in 2017). In 2017, when total surface sown per plant species differed, as recommended by Zuur et al. (2013), the rate at which events occur in areas of different sizes was included in the model to offset these variations, as the surfaces sown may have different levels of attractiveness to chrysopids.

The correlation between chrysopid abundance and the potential prey Acari, Aphididae, Collembola and Thysanoptera was tested each year using the Pearson test.

All statistical analyses were carried out using R software version 3.5.0 (R Development Core Team, 2017), and the "*agricolae*" package (De Mendiburu, 2014) was used for the Kruskal-Wallis test.

7.5. Results

7.5.1. *Growth of plant species*

A total of 30 out of 42 plant species sown, which covered over 70% of the surface sown, recorded adequate growth in both years of the study (Table 1). In 2016, only 5 out of the 40 plant species sown, belonging to four botanical families, which showed unsatisfactory vegetative development, were not sampled: *Tuberaria guttata, Helianthemum ledifolium* (Cistaceae*), Anarrhinum bellidifolium* (Veronicaceae)*, Aegilops geniculata* and *Aegilops triuncialis* (Poaceae). In 2017, two of the 22 plant species belonging to the Fabaceae family (*Medicago polymorpha* and *Medicago orbicularis)* did not grow correctly. Two other plant species, *Capsella bursa-pastori* in 2016 and *Vaccaria hispanica* in 2017, which failed to bloom during the sampling period, were not included in the sample (Table 1).

7.5.2. *Arthropods collected*

An overall evaluation of the 42,130 arthropods collected (21,723 in 2016 and 20,408 in 2017) showed that the Neuroptera Order of insects belonging exclusively to the Chrysopidae family, was a tiny minority in both sampling years (0.27% in 2016, 0.86% in 2017) as compared to other taxa groups in natural enemy assemblages such as parasitoids (8.56% in 2016, 5.04% in 2017), predators belonging to the Suborder Heteroptera (1.7% in 2016, 5.9% in 2017) and the Order Araneae (0.94% in 2016, 1.25% in 2017). The mean abundance of Chrysopidae species collected each year was also significantly higher in 2017 (1.54±0.17 individuals/replica, n=114) than in 2016 (0.28±0.06 individuals/replica, n=204) (Kruskal-Wallis Chisq= 68.83, p<0.001). Among the potential prey available for natural enemies, the most abundant taxa of the total arthropods captured were the Orders Collembola (40.43% in 2016, 31.97% in 2017) and Thysanoptera (21.26% in 2016, 28.99% in 2017) (Table 2). The low and irregular abundance of the Order Acari ranged from zero in many plant species to 75.5±37.3 individuals/replica. Members of the Aphididae family, which were more abundant in 2017, were present in all plant species (Supplementary Material, Table S2).

Table 2. Total abundance and frequency (%) of taxa collected in 2016 and 2017.

Taxa	2016		2017	
	No. of specimens	**%**	**No. of specimens**	**%**
Acari	111	0.51	2,035	9.97
Aphididae	326	1.5	1,276	6.25
Araneae	205	0.94	255	1.25
Apidae	25	0.12	63	0.31
Cicadellidae	824	3.79	159	0.78
Coleoptera	1,228	5.65	435	2.13
Collembola	8,782	40.43	6,525	31.97
Diptera	3,184	14.66	863	4.23
Embioptera	2	0.01	0	0
Formicidae	75	0.35	176	0.86
Heteroptera	369	1.7	1,203	5.89
Lepidoptera	20	0.09	73	0.36
Neuroptera	58	0.27	176	0.86
Orthoptera	2	0.01	0	0
Parasitoids	1,859	8.56	1,029	5.04
Psocoptera	19	0.09	204	1
Syrphidae	16	0.07	19	0.09
Thysanoptera	4,618	21.26	5,917	28.99
Total	**21,723**		**20,408**	

7.5.3.*Chrysopid species*

Chrysopid species diversity was low, with all 46 adults recorded in 2016 identified as belonging to the *C. lucasina* species (27 females and 19 males; Table 3). 95% (63) of specimens collected in 2017 were identified as *C. lucasina* (44 females and 19 males), with 5% (3) of specimens identified as *Chrysoperla pallida* (Henry et al., 2002) females (Table 5).

7.5.4.*Plants visited by chrysopid species*

With regard to the 36 plant species sampled, during the two years of the study, 28 were visited by chrysopids at the preimaginal and adult stages. Of the eight plants not visited, seven were only sown in 2016, and *Calendula arvensis* was the only plant species present during the two years of the study (Table 1). Of the 28 species visited by chrysopids, 21 were visited by C. *lucasina* adults*,* while *C. pallida* adults were recorded in the plant species *Echium plantagineum*, *Glebionis segetum* and *Trifolium hirtum,* with *C. lucasina* only absent from *T. hirtum* (Tables 3, 5).

Table 3. Plants presenting chrysopids in replica plot sampling (2016). Rows in bold indicate plant species with above-average mean chrysopid abundance. The only chrysopid species captured was *C. lucasina*.

Family	***Species***	***Replica plots with chrysopids (maximum=6)***	***Total chrysopids (mean±SE)***	***Adults***	***Preimaginal stages***	***C. lucasina***	
						female	***male***
Apiaceae	***Tordylium maximum L.***	**2**	**2 (0.3±0.2)**	**1**	**1**	**1**	**0**
Asteraceae	*Crepis capillaris (L.) Wall.*	1	1 (0.2±0.2)	1	0	0	1
Asteraceae	*Glebionis segetum L.*	1	1 (0.2±0.2)	1	0	1	0
Boraginaceae	*Echium plantagineum L.*	1	1 (0.2±0.2)	1	0	1	0
Brassicaceae	***Biscutella auriculata L.***	**4**	**7 (1.2±0.5)**	**5**	**2**	**4**	**1**
Brassicaceae	***Moricandia moricandioides Boiss.***	**4**	**17 (2.8±1.5)**	**16**	**1**	**5**	**11**
Caryophyllaceae	***Silene colorata Poir.***	**4**	**5 (0.8±0.3)**	**3**	**2**	**3**	**0**
Caryophyllaceae	***Vaccaria hispanica (Mill.) Rauschert***	**3**	**5 (0.8±0.4)**	**5**	**0**	**3**	**2**
Fabaceae	***Medicago orbicularis (L.) Bartal.***	**2**	**2 (0.3±0.2)**	**2**	**0**	**1**	**1**
Dipsacaceae	*Scabiosa atropurpurea L.*	1	1 (0.2±0.2)	1	0	1	0
Fabaceae	***Trifolium angustifolium L.***	**1**	**2 (0.3±0.3)**	**0**	**2**	**0**	**0**
Fabaceae	*Trifolium stellatum L.*	1	1 (0.2±0.2)	0	1	0	0
Lamiaceae	*Cleonia lusitanica L.*	1	1 (0.2±0.2)	1	0	1	0
Poaceae	*Bromus hordeaceus L.*	1	1 (0.2±0.2)	0	1	0	0
Poaceae	*Cynosurus echinatus L.*	1	1 (0.2±0.2)	1	0	1	0
Poaceae	*Hordeum murinum, subsp. leporinum L.*	1	1 (0.2±0.2)	0	1	0	0
Poaceae	***Lolium multiflorum Lam.***	**3**	**5 (0.8±0.4)**	**5**	**0**	**3**	**2**
Poaceae	*Trachynia distachya (L.) Beauv.*	1	1 (0.2±0.2)	1	0	0	1
Ranunculaceae	***Nigella damascena L.***	**2**	**2 (0.3±0.2)**	**2**	**0**	**2**	**0**

7.5.5. *Sampling in replica plots (2016)*

Chrysopids were collected from 19 of the 34 plant species sampled in 2016, with nine of these plants presenting abundant chrysopids (up to their annual average abundance), and the plant species with the highest mean chrysopid abundance were *Moricandia moricandioides* and *Biscutella auriculata*. On the other hand, ten plant species, from which only one chrysopid specimen was collected, showed below annual average mean abundance (Table 1, 3).

In the 2016 sampling, the GLM model showed that chrysopid abundance depends solely on plant species and not prey abundance (Table 4). However, the high variability in chrysopid abundance in replica plots per plant species meant that pairwise comparisons of plant species did not differ significantly. In addition, although we found potential prey for chrysopids in all plant species (Supplementary Material, Table S1), chrysopid abundance did not correlate closely with potential prey (Pearson test; r<0.04) (Supplementary Material, Table S2).

Table 4. Results of generalized linear model (GLM) for each year of the study, degree of freedom (d.f.), Chi-squared value (χ^2) and *p* value were obtained by Anova analysis of GLM deviance. Significance of variables in the model is indicated as follows: *** *P* <0.001, ***P* <0.01, and * *P* <0.05.

Year	Variables	Estimate	χ^2	d.f.	*p* value	
2016	Plant species		44.707	18	<0.001	***
	Prey abundance	1.907e-04	0.003	1	0.953	
2017	Plant species		85.338	17	<0.001	***
	Prey abundance	3.481e-03	12.645	1	<0.001	***

7.5.6. *Sampling in replica samples (2017)*

In 2017, 18 of the 19 plant species were visited by chrysopids (Table 1, 5), eight of which exceeded annual average chrysopid abundance, with *B. auriculata* recording the highest mean abundance of captured chrysopids. On the other hand, 11 plant species showed a below annual average mean abundance of captured chrysopids (1 to 8 chrysopids).

The GLM model demonstrated that chrysopid abundance in 2017 depended on plant species and prey abundance (Table 4). Pairwise comparison of plant species with chrysopids showing above-average annual mean abundance (Figure 2) highlighted two significantly different groups. The first group was composed of *B. auriculata*, *B. officinalis*, *Silene colorata* and *Crepis capillaris*, while the second group comprised *E. plantagineum*, *P. rhoeas* and *Nigella damascena*, although no differences between plant species within each group were observed. All plants in both groups showed abundant potential prey (Supplementary Material, Table S3), with only the abundance of Thysanoptera being positively correlated with that of chrysopids (Pearson test; r<0.15, *p*<0.05) (Supplementary Material, Table S2).

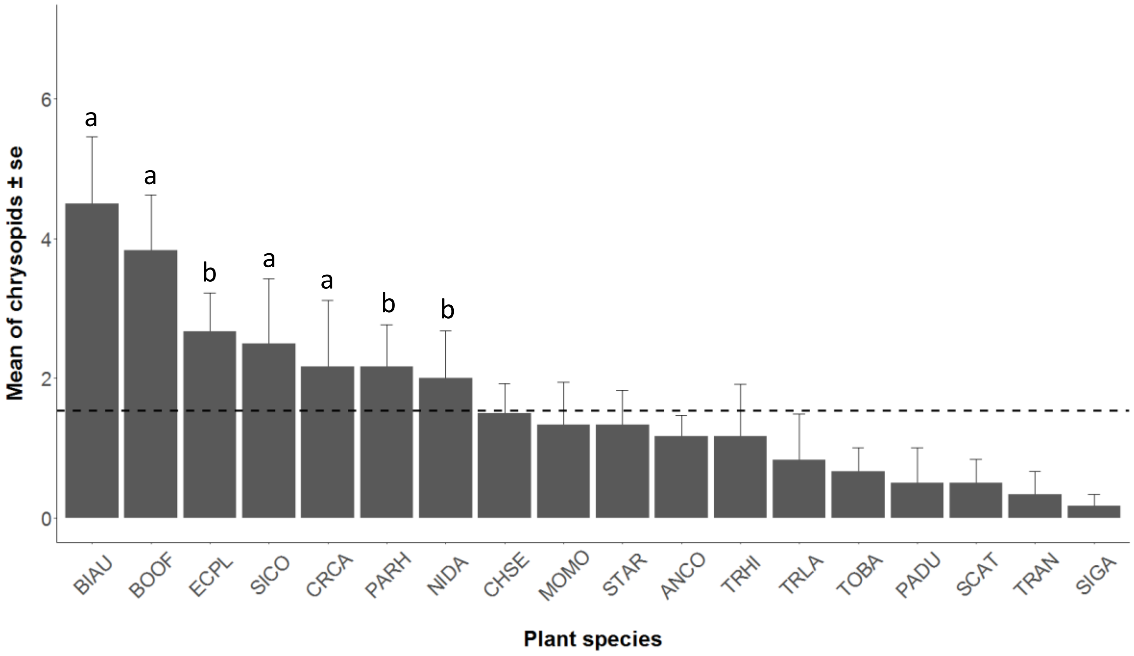

Figure 2. Chrysopid abundance (mean±SE) for each plant species studied (n=6) in 2017. Lower case denotes significant pairwise Tukey´s test differences between plant species studied. Significant differences are shown only when the mean of abundance is higher than average annual chrysopid abundance in 2017 (indicated by a horizontal dashed line). For plant species codes, see Table 1.

7.5.7. *Use of plant species as habitats for reproduction, feeding and/or resting*

In the 28 plant species visited by chrysopids, 23 eggs, 98 larvae of different ages and 112 adults were found. Of these, 22 contained chrysopids at the preimaginal stage, indicating that these plants were used for reproduction. Adults were found in 22 plant species, which were possibly used for feeding and/or rest. These activities, together with reproduction, occurred in 16 of the plant species visited (Table 3, 5).

Table 5. Plants presenting chrysopids in replica samples (2017). Rows in bold indicate plant species with above-average annual mean chrysopid abundance.

Family	***Species***	***Replica samples with chrysopids (maximum = 6)***	***Total chrysopids (mean±SE)***	***Adults***	***Preimaginal stages***	***C. lucasina***		***C. pallida***
						females	***males***	***females***
Asteraceae	*Anthemis cotula L.*	5	7 (1.2±0.3)	0	7	0	0	0
Asteraceae	***Crepis capillaris (L.) Wall.***	**4**	**13 (2.2±1)**	**4**	**9**	**2**	**2**	**0**
Asteraceae	*Glebionis segetum L.*	5	9 (1.5±0.4)	3	6	2	0	1
Asteraceae	*Tolpis barbata (L.) Gaertn.*	3	4 (0.7±0.3)	0	4	0	0	0
Boraginaceae	***Borago officinalis L.***	**6**	**23 (3.8±0.8)**	**1**	**22**	**1**	**0**	**0**
Boraginaceae	***Echium plantagineum L.***	**6**	**16 (2.7±0.6)**	**5**	**11**	**3**	**1**	**1**
Brassicaceae	***Biscutella auriculata L.***	**6**	**27 (4.5±1)**	**15**	**12**	**11**	**4**	**0**
Brassicaceae	*Moricandia moricandioides Boiss.*	4	8 (1.3±0.6)	3	5	2	1	0
Caryophyllaceae	***Silene colorata Poir.***	**4**	**15 (2.5±0.9)**	**12**	**3**	**7**	**5**	**0**
Caryophyllaceae	*Silene gallica L.*	1	1 (0.2±0.2)	0	1	0	0	0
Dipsacaceae	*Scabiosa atropurpurea L.*	2	3 (0.5±0.3)	2	1	1	1	0
Fabaceae	*Trifolium angustifolium L.*	1	2 (0.3±0.3)	2	0	2	0	0
Fabaceae	*Trifolium hirtum All.*	2	7 (1.2±0.8)	1	6	0	0	1
Fabaceae	*Trifolium lappaceum L.*	2	5 (0.8±0.7)	1	4	0	1	0
Lamiaceae	*Stachys arvensis L.*	4	8 (1.3±0.5)	5	3	4	1	0
Papaveraceae	*Papaver dubium L.*	1	3 (0.5±0.3)	1	2	1	0	0
Papaveraceae	***Papaver rhoeas L.***	**6**	**13 (2.2±0.6)**	**9**	**4**	**7**	**2**	**0**
Ranunculaceae	***Nigella damascena L.***	**6**	**12 (2±0.7)**	**2**	**10**	**1**	**1**	**0**

7.6. Discussion

Plant selection was highly effective, as 36 of the 42 species sown showed satisfactory vegetative development, with blossoming occurred, before that of olive trees.

The abundance of the Neuroptera taxa was found to be much lower than that of other predator arthropods such as spiders and coleopteran which is in line with the findings of other authors (Franin et al., 2016; Mignon et al., 2003; Silva et al., 2010). More specifically, different studies have also found that chrysopids, which are among the most widespread and active predators, are the least abundant predators of aphids as compared to ladybeetles and hoverflies (Bertolaccini et al., 2011; Hatt et al., 2017).

With regard to chrysopid species richness, only two species, *C. lucasina* and *C. pallida*, were present in our study. *C. lucasina* was the most abundant species collected, which is probably due to its wide holomediterranean distribution, effective adaptation to high temperatures compared to other species (Thierry et al., 1996) and its preference for spring-flowering herbaceous plants (Paulian, 2001; Villenave et al., 2005). On the other hand, the limited presence of *C. pallida* could be related to its preference for trees both inside and on the edge of forests despite its visits to herbaceous plants (Duelli et al., 2002; Monserrat, 2016).

Fluctuations in the abundance of chrysopid populations over the two years studied could be explained by changes in weather conditions and/or potential prey (Szentkirályi, 2001a) or by the possible differential attractiveness of the surfaces sown during the two years of the study. In the study area, the warmer and drier spring of 2017 may have favoured *C. lucasina* populations with their xerophile tendencies. In addition, the colder winter of 2015 as compared to 2016 may have raised adult chrysopid mortality, which, as occurs in other zones, would affect the spring populations of this species (Villenave, 2006). With regard to prey, the larger presence of aphids in 2017 could have benefited *C. lucasina* populations given their marked aphidophagous nature (Canard et al., 1984).

With regard to the 36 plant species sampled during the two-year study, 28 were visited by chrysopids. Although plant species was observed to be an important factor affecting the presence of chrysopids, this variable did not have a clear pattern probably due to a mix of factors related to inter-annual variations in arthropod populations. For example, in 2016, the absence of a marked preference for any of the plant species is explained by the highly variable abundance of the chrysopids captured in each replica plot. Nevertheless, with the large size of areas sown and higher chrysopid abundance in 2017, the plant species *B. auriculata*, *B. officinalis*, *S. colorata* and *C. capillaris* were found to be particularly attractive to chrysopids. Although the selection of plants by *C. carnea s.l.* has not been reported due to its generalist and opportunistic behaviour (Duelli, 1987), adult lacewings should clearly be associated with or have a preference for certain plant species when seeking pollen, nectar or prey, given that their presence and availability stimulate colonization (Canard et al., 1984; Devetak and Klokocovnik, 2016; Ruby et al., 2011). Availability of vegetal species plays a major role in visits by chrysopids (Villenave et al., 2006), however we studied in both years the plant species under similar conditions (phenology, location and sampling date). Other factors that may affect chrysopid plant visits is flower morphology and pollen production, as *C. carnea s.l.* is known to be most attracted to umbeliferous members of the Asteraceae family and other plant species with exposed nectaries. However, the width of its head and thorax and length of its antennae may limit the number of flowers capable of providing nectar, which is extracted from extrafloral nectaries in some species (Nave et al., 2016; Van Rijn, 2012; Wäckers and Van Rijn, 2012). With its consumption apparently essential for *C. carnea s.l.* reproduction (Villa et al., 2016), the availability of pollen may explain the large number of visits to species such as *B. auriculata* and *P. rhoeas* despite their reported low nectar production (Hicks et al., 2016; Hidalgo and Cabezudo, 1995).

Although *C. lucasina* adults are known to randomly forage on a broad plant species, only consume the pollen of some plant species (Villenave, 2006; Villenave et al., 2006). *C. arvensis* plant species were not visited by chrysopids during the two years study, which could be due to a lack of attraction, or aversion, to the plant, a phenomenon observed in other chrysopid species (Villa et al., 2016; Villenave, 2006). On the other hand, the *C. lucasina* preference for low vegetation and large patches of flowering plants (Villenave, 2006) led chrysopid adults to visit 22 of the plant species sampled belonging to 11 botanical families, which could constitute an important source of food for chrysopids.

Potential prey residing in the plants constitutes another resource for chrysopids. Prey and/or their honeydews, produced by some prey, emit kairomones which attract *C. carnea s.l.* adults, enabling them to locate the plant and to find optimal oviposition sites (McEwen et al., 1993). Our study showed that abundant prey (Acari, Aphididae, Collembola and Thysanoptera) were available on the plant species sampled, although chrysopid abundance was correlated with prey abundance only in 2017. We found statistical evidence that, among available potential prey, thrips positively correlate with chrysopid abundance. Aphids are known to be a favourite prey of *C. carnea s.l.* larvae in different crops;

preoviposition flights in spring are also associated with the presence of aphid colonies, although the polyphagous nature of larvae means that very different types of prey are sought and consumed (Duelli, 2001; Villenave et al., 2005). Bertolaccini et al. (2011) have also reported the presence of *C. carnea* in numerous spontaneous plants, such as *Echium vulgare* and *C. capillaris,* which had no aphids.

The plant species most visited by chrysopids in our study belonged to four families: *B. auriculata* (Brassicaceae), *B. officinalis* (Boraginaceae), *S. colorata* (Caryophyllaceae) and *C. capillaris* (Asteraceae), which could be related to the feeding preference of *C. carnea, C. lucasina* and *Chrysoperla affinis* (Stephens, 1836) on these botanical families' previously reported by Denis and Villenave (2009). Our study shows that four species belonging to the genus *Trifolium* (Fabaceae) were visited by chrysopids as habitats both for reproduction and probably also for feeding, in agreement with findings of Villenave et al. (2005) as pollen from this family has been detected on the diverticulum of *C. carnea s.l.*. Moreover some different species of Brassicaceae and Fabaceae, also prevent erosion and boost crop fertility and biofumigation, being commonly used as vegetal cover in olive groves (Gálvez et al., 2016). Thus the selection of certain plant species could enhance different ecosystem services not only improve biological control.

Knowledge of the function of habitats as reproduction and feeding sites is vital for improving the sustainability of ecosystem services (Bianchi et al., 2013). In our study, the preimaginal stage of chrysopids present in different plant species show that 22 species were used by chrysopids for reproduction in spring, and 22 species were used for feeding or rest, a finding which is of considerable importance for managing the survival and maintenance of these populations. Chrysopids were found to perform both activities in 16 plant species; as most adults are female, they usually oviposit where food is available, behaviour which has been observed in *C. carnea s.l.* (Duelli, 1984), and specifically on species such as *Trifolium pratense*, *Centaurea cyanus* and *P. rhoeas* (Franin et al., 2016).

The results obtained in our study enabled us to determine the relationship between chrysopids and the native plant species selected, although flower visitation rates, which are relative measures, depend on the presence of other flowering species, competition with other flower visitors and on previous experience (Wäckers and Van Rijn, 2012). Thus, in future research, it will be important to determine their suitability in terms of actual fitness benefits in relation to survival and reproduction (Wäckers and Van Rijn, 2012). In this regard, different spontaneous Mediterranean flowering plants have been shown in the laboratory to improve *C. carnea s.l.* adult survival rates, while pollen consumption appears to be essential for reproduction (Villa et al., 2016). The sugar profile and content of Mediterranean flowering plants as a food resource for *C. carnea* adults have been evaluated, with trehalose content in pollen and nectar playing a key role in *C. carnea* fecundity and longevity (Gonzalez et al., 2016).

Given the practical aims of our study, another factor to be considered is that the plants selected do not transmit diseases to crops and/or have a positive impact on their pest populations. In this study, the species *M. moricandioides* and *Hordeum murinum* were observed to be used by chrysopids to feed and/or rest and reproduce. However, they can present a certain level of susceptibility to the bacteria causing important olive tree disease *Verticillium dahliae* or act as asymptomatic visitors, which favours the maintenance and multiplication of *V. dahliae* populations in olive plantations (Bejarano-Alcazar et al., 2004). Likewise, species such as *Malva sylvestris* and *E. plantagineum* have been shown in the laboratory to positively affect the longevity and/or reproduction of *Prays oleae,* one of the main pests in olive groves (Nave, 2016; Villa et al., 2017).

7.7. Conclusion

In summary, our results demonstrate that 28 native plants with adequate vegetative growth are used by chrysopids as habitats to reproduce, feed and/or rest. The adults captured were *C. lucasina* and *C. pallida,* with a predominance of the former. In 2017, visits by chrysopids were determined by the plant species and the prey present in these species; among the potential prey available, thrips were shown to have a positive effect on chrysopid abundance. Of the eight plant species, with above-average chrysopid abundance, *B. auriculata*, *B. officinalis*, *S. colorata* and *C. capillaris* were the most attractive to these lacewings. Given the attraction of chrysopids, the effect of pests and disease on olive groves and satisfactory vegetative growth, in our view, *B. auriculata*, *B. officinalis*, *S. colorata*, *C. capillaris, N. damascena* and *P. rhoeas* are among the best species to restore ground cover for woody Mediterranean crops such as olive trees and to increase populations of these key natural enemies of olive pests (graphical abstract).

7.8. References

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7.1. Supplementary materials

Table S1. Total abundance (mean±SE) in 2016 of potential chrysopid prey collected from each plant species (n= 6).

Family	***Species***	***Acari***	***Aphididae***	***Collembola***	***Thysanoptera***
Apiaceae	*Tordylium maximum L.*	0	3 (0.5±0.3)	5 (0.8±0.4)	28 (4.7±1.8)
Asteraceae	*Crepis capillaris (L.) Wall.*	19 (3.2±1.2)	4 (0.7±0.3)	171 (28.5±10)	536 (89.3±28.1)
Asteraceae	*Glebionis segetum L.*	0	4 (0.7±0.5)	14 (2.3±1.1)	194 (32.3±6.5)
Boraginaceae	*Echium plantagineum L.*	0	0	166 (27.7±17.7)	57 (9.5±3.5)
Brassicaceae	*Biscutella auriculata L.*	0	1 (0.2±0.2)	31 (5.2±2.5)	648 (108±37.4)
Brassicaceae	*Moricandia moricandioides Boiss.*	0	2 (0.3±0.3)	0	137 (22.8±5.8)
Caryophyllaceae	*Silene colorata Poir.*	10 (1.7±1.3)	2 (0.3±0.2)	72 (12±2.4)	86 (14.3±4)
Caryophyllaceae	*Vaccaria hispanica (Mill.) Rauschert*	10 (1.7±0.5)	2 (0.3±0.2)	10 (1.7±0.8)	154 (25.7±11.3)
Dipsacaceae	*Scabiosa atropurpurea L.*	0	1 (0.2±0.2)	237 (39.5±12.1)	33 (5.5±2.4)
Fabaceae	*Medicago orbicularis (L.) Bartal.*	0	28 (4.7±0.9)	26 (4.3±1.7)	229 (38.2±16.4)
Fabaceae	*Trifolium angustifolium L.*	10 (1.7±0.6)	4 (0.7±0.7)	202 (33.7±9)	38 (6.3±2.7)
Fabaceae	*Trifolium stellatum L.*	7 (1.7±0.5)	22 (3.7±1.9)	153 (25.5±3.3)	66 (11±3.3)
Lamiaceae	*Cleonia lusitanica L.*	0	2 (0.3±0.3)	60 (10±1.4)	16 (2.7±1)
Poaceae	*Bromus hordeaceus L.*	2 (0.3±0.3)	15 (2.5±0.9)	334 (55.7±10.7)	146 (24.3±9.5)
Poaceae	*Cynosurus echinatus L.*	0	28 (4.7±0.8)	175 (29.2±10.6)	165 (27.5±5.9)
Poaceae	*Hordeum murinum, subsp. Leporinum L.*	0	25 (4.2±1.1)	597 (99.5±28.4)	328 (54.7±12.3)
Poaceae	*Lolium multiflorum Lam.*	0	13 (2.2±0.8)	903 (150.5±42.2)	108 (18±2.6)
Poaceae	*Trachynia distachya (L.) Beauv.*	0	8 (1.3±0.8)	849 (141.5±20.9)	87 (14.5±6.7)
Ranunculaceae	*Nigella damascena L.*	15 (2.5±0.6)	11 (1.8±0.9)	161 (26.8±9.4)	81 (13.5±3.1)

Table S2. Correlation between potential prey and chrysopid abundance in both years studied (2016, 2017). Significance of variables in the model is indicated as follows: *** *P* <0.001, ***P* <0.01, and * *P* <0.05.

Year	Potential prey	Pearson correlation coefficient	t-test statistic	Degree of freedom (d.f.)	*p* value
2016	Acari	0.008	0.12	202	0.905
	Aphididae	-0.08	-1.191	202	0.235
	Collembola	-0.06	0.85	202	0.4
	Thysanoptera	0.04	0.064	202	0.547
2017	Acari	0.148	1.585	112	0.116
	Aphididae	-0.071	-0.754	112	0.45
	Collembola	0.035	0.37	112	0.712
	Thysanoptera	0.414	4.811	112	<0.001

Table S3. Total abundance (mean±SE) in 2017 of potential chrysopid prey collected from each plant species (n= 6).

Family	***Species***	***Acari***	***Aphididae***	***Collembola***	***Thysanoptera***
Asteraceae	*Anthemis cotula L.*	199 (33.2±16.8)	144 (24±8.8)	481 (80.2±20.9)	451 (75.2±13.2)
Asteraceae	*Crepis capillaris (L.) Wall.*	107 (17.8±9.31)	29 (4.8±0.8)	698 (116.3±21.9)	650 (108.3±25.5)
Asteraceae	*Glebionis segetum L.*	0	120 (10±6.7)	14 (2.3±1.1)	631 (105.2±13.7)
Asteraceae	*Tolpis barbata (L.) Gaertn.*	189 (31.5±15)	24 (4±1.3)	286 (47.7±10.1)	167 (27.8±9.5)
Boraginaceae	*Borago officinalis L.*	110 (18.3±9.3)	91 (15.2±3.3)	103 (17.2±6.8)	168 (28±4.3)
Boraginaceae	*Echium plantagineum L.*	0	30 (5±2.1)	155 (25.8±13.1)	706 (117.7±22.5)
Brassicaceae	*Biscutella auriculata L.*	0	11 (1.8±1.1)	67 (11.2±5.3)	983 (163.8±49.6)
Brassicaceae	*Moricandia moricandioides Boiss.*	4 (0.7±0.7)	10 (1.7±0.8)	15 (2.5±0.9)	492 (82±10.1)
Caryophyllaceae	*Silene colorata Poir.*	8 (1.3±1.3)	15 (2.5±1.5)	274 (45.7±21.9)	155 (25.8±11.6)
Caryophyllaceae	*Silene gallica L.*	174 (29±16.7)	29 (4.8±1.9)	12 (2±1.2)	158 (26.3±5.5)
Fabaceae	*Trifolium angustifolium L.*	2 (0.3±0.3)	208 (34.7±15.7)	644 (107.3±32.5)	140 (23.3±5.6)
Dipsacaceae	*Scabiosa atropurpurea L.*	0	37 (6.2±3.5)	345 (57.5±8.9)	55 (9.2±1.1)
Fabaceae	*Trifolium hirtum All.*	403 (67.2±34.5)	88 (14.7±3.1)	140 (23.3±13.6)	46 (7.7±1.5)
Fabaceae	*Trifolium lappaceum L.*	386 (64.3±31.8)	284 (47.3±10)	1,200 (200±74)	136 (22.7±9.1)
Lamiaceae	*Stachys arvensis L.*	0	59 (9.8±4.7)	440 (73.3±16.7)	113 (18.8±2.7)
Papaveraceae	*Papaver dubium L.*	0	8 (1.3±0.7)	4 (0.7±0.3)	27 (4.5±2.6)
Papaveraceae	*Papaver rhoeas L.*	0	12 (2±0.8)	18 (3±1.6)	353 (58.8±11.3)
Ranunculaceae	*Nigella damascena L.*	453 (75.5±37.3)	17 (2.8±1.2)	1,629 (271.5±31.4)	413 (68.8±15.9)

Discusión General

Diversos aspectos de la ecología de los crisópidos han sido abordados en la presente tesis en relación al incremento y conservación de sus poblaciones en las infraestructuras ecológicas del agroecosistema del olivar. La intensificación de las prácticas agrícolas han generado un declive de la biodiversidad funcional y los servicios ecosistémicos, como por ejemplo el control biológico de plagas (Altieri and Nicholls 2004, Zhang et al. 2007). La actual política agraria común europea demanda que se garantice una mayor protección del medio ambiente y la biodiversidad, de modo que se deben buscar prácticas agrícolas más sostenibles y respetuosas con el medio ambiente. Además, la Unión Europea establece entre los principios generales de la gestión integrada de plagas, la utilización de infraestructuras ecológicas dentro y fuera de los cultivos para la protección y mejora de los organismos beneficiosos importantes (European Union 2009). Por tanto, el valor de la contribución científica de esta tesis radica en que los conocimientos adquiridos podrán ser aplicados en el diseño de estrategias para la conservación de la biodiversidad y el control biológico por conservación en el contexto de la gestión integrada de plagas en el olivar.

8.1. Influencia de las especies arbóreas adyacentes al olivar sobre la familia Chrysopidae

A fin de evaluar y analizar adecuadamente el papel de las infraestructuras ecológicas, denominadas también como hábitats seminaturales (HSNs), sobre la comunidad de crisópidos asociada al agroecosistema del olivar, se optó por centrar los estudios sobre especies vegetales concretas y frecuentes en los HSNs del olivar (almendro, encina y pino), en una amplia escala temporal y espacial. Las metodologías de muestreo fueron seleccionadas en función de las fases de desarrollo de los crisópidos que se querían colectar y que ya habían sido probadas en estudios previos (Alrouechdi 1980, Campos 1986, Porcel 2012): observación directa para los huevos (capítulo 1), bandas de cartón para las larvas y prepupas/pupas (capítulo 2), y succión para los adultos (capítulo 3), aunque con la succión también se capturaron larvas.

En el capítulo 1 observamos la dificultad que entraña realizar colectas de puestas de crisópidos cuando su abundancia es baja, y no fue hasta el verano (agosto exactamente), cuando las densidades ya eran elevadas y pudimos realizar los muestreos. De hecho estudios previos señalan el verano como un período de altas densidades de crisópidos (Campos 1989, Pantaleoni et al. 1993, Marín 1994). Nuestros resultados mostraron que no hubo diferencias significativas respecto al número de puestas entre almendro, encina, olivo y pino por lo que deducimos que las hembras de los crisópidos encuentran estructuras vegetales igualmente adecuadas para oviponer y/o para el desarrollo de su descendencia (abundancia de presas) en todas las especies arbóreas. Ambos factores han sido consideramos importantes para determinar el comportamiento de los crisópidos frente a la oviposición (Duelli 1984, Nakamura et al. 2000). Otro aspecto de interés de este estudio es que, en general, los lugares de puesta más abundantes son el borde y el haz de la hoja y la forma aislada es el modo de puesta con mayor frecuencia que podemos encontrar. Ambos factores, modo y lugar de puesta, van a estar relacionados con la comunidad de crisópidos del biotopo estudiado, de forma que sólo dos de las nueve especies identificadas, deponen sus huevos en forma de racimo (*Pseudomallada marianus* y *Pseudomallada flavifrons*). *Pseudomallada prasinus* fue la especie más generalista al encontrar sus puestas en almendro, encina, olivo y pino.

Aunque en el estudio del capítulo 1 no llegamos a detectar diferencias en las puestas de los crisópidos a nivel de especie de árbol, en un estudio a largo plazo, con mayor perspectiva y centrado en las fases juveniles (larva, prepupa/pupa) (capítulo 2), observamos que su abundancia en el almendro, encina y olivo eran mayores que en el pino. Otro aspecto destacable en nuestro estudio es que el parasitismo fue el mayor factor de reducción de las poblaciones en esta segunda fase desarrollo y es muy similar a estudios previos en el sur de España en el olivar (Campos 1986). Además a nivel de especie de árbol, el olivo registró las menores tasas de parasitismo comparado con estudios previos (Neuenschwander and Michelakis 1980, Alrouechdi et al. 1981, Campos 1986) y considerablemente inferior que las registradas sobre el almendro, encina y pino. Si a esto le añadimos los bajos valores de juveniles depredados, los juveniles muertos por factores desconocidos y aquellos que completaron su desarrollo hasta adulto, convierten al olivo en la especie arbórea con mayor contribución de crisópidos viables para la próxima generación.

La comunidad de crisópidos en nuestro biotopo, identificados a partir de los adultos emergidos de los estadios juveniles, estuvo compuesta por diez especies, resultando también *P. prasinus* la más abundante a lo largo de todo el período de estudio, además observamos que hibernó en el almendro, encina y olivo. Mientras que el pino, se colectaron cinco especies de entre las cuales *C. mediterranea* fue la dominante con una elevada especificidad por esta especie de árbol.

El complejo parasitario de nuestro biotopo lo compusieron tres parasitoides primarios (*Baryscapus impeditus*, *Helorus ruficornis* y *Isodromus puncticeps*) y dos especies (*Gelis ilicicola* y *Perilampus minutalis*) catalogadas como parasitoides primarios pero que además pueden actuar como hiperparasitoides. *B. impeditus*, fue el más abundante, preferentemente asociado a los juveniles de *Chrysoperla mediterranea*, *Chrysoperla lucasina* y *Chrysopa pallens*, las cuales fueron recolectadas sobre almendro y pino. La segunda especie en importancia fue *H. ruficornis*, que estuvo preferentemente asociado a las especies de los géneros *Pseudomallada* (*P. picteti*, *P. flavifrons* y *P. prasinus*) y *Cunctochrysa* (*C. baetica*), y que fueron colectadas sobre la encina.

Tras conocer los factores de reducción de las fases juveniles de la familia Chrysopidae, el último paso fue determinar la atractividad de la fase adulta por las diferentes especies arbóreas de los HSNs (en los mismos olivares ecológicos del capítulo 2), durante el período de mayor actividad para los crisópidos (abril a octubre) y tratar de identificar los factores responsables de su abundancia (capítulo 3). Se capturaron 17 especies, siendo las cuatro más abundantes *P. prasinus*, *C. lucasina*, *C. mutata* y *C. pallida*. Además, se comprobó que la encina fue el lugar de refugio preferente durante todo el período de estudio porque registró la mayor abundancia de crisópidos, seguido por el almendro (con picos de abundancias en el mes de junio), y por el olivo (con abundancias destacadas en los meses de septiembre y octubre). Esta variación temporal podría deberse a las diferentes especies de crisópidos que cada especie arbórea acoge. Si comparamos la riqueza de especies capturadas mediante succión con la de las bandas de cartón (10 especies), se podría pensar que no todos las especies en fase adulta utilizan el almendro, encina, olivo o pino como lugar de reproducción.

P. prasinus mostró una clara preferencia por la encina, aunque también fue capturada en almendro, olivo y de forma marginal en el pino, este resultado es comparable al obtenido en el capítulo 2. Consideramos que *P. prasinus* encuentra mayor refugio en la forma y densidad de la copa de la encina frente a las del almendro, olivo o pino. Además, aunque luego hiberne en las cuatro especies de árbol, las fases juveniles fueron abundantes sobre el olivo (capítulo 2). Por otro lado, *C. lucasina* es atraída por las cubiertas, que tienen todos los olivares de estudio, y se desplazó también a la vegetación adyacente, en nuestro caso almendro, encina y pino, para utilizarla como lugar de refugio y/o alimentación, especialmente en aquellos meses en los que la cubierta había sido segada, tal y como ya apuntaba Duelli (2001).

En el caso de *C. mutata* y *C. pallida*, la atracción que tienen por el olivo en los meses de otoño podría deberse a que en él encuentran las melazas con las que elaborar los lípidos con los cuales afrontar el invierno (Hagen and Tassan 1972, Lemesle et al. 1998).

La metodología de muestreo en este capítulo 3 permitió capturar, además de individuos en fase adulta, individuos en fase larvaria. Aunque las mayores capturas se registraron en el olivo, especialmente en los meses de junio y julio, justificable por su atracción por la plaga del olivo *Prays oleae* (Ramos et al. 1984). A pesar de ello, no hemos registrado una causa directa entre la abundancia de larvas y adultos con respecto al porcentaje de huevos depredados de *P. oleae* en la generación antófaga y carpófaga. Además, hemos detectado una falta de asociación entre la composición botánica en las cubiertas y en la linde-mancha de vegetación con respecto a la abundancia de crisópidos en los olivares estudiados. Esta falta de asociación la justificamos por la heterogeneidad botánica entre los olivares, dado que estamos tratando de comparar olivares de diferentes extensiones y densidades de HSNs (manchas-lindes), estas dos variables no están intrínsecamente correlacionadas tal y como se refleja en estudios previos (Fahrig 2013, Martin et al. 2019). Por otro lado hay que tener en cuenta el carácter oportunista y la gran capacidad de dispersión de los crisópidos, que desemboca en el uso de la vegetación existente sin un patrón determinado, algo ya observado para las cubiertas por Villenave et al. (2005).

8.2. Atractividad de especies herbáceas nativas para los crisópidos y su posible uso como cubierta en el olivar

Las cubiertas vegetales ya sea de especies espontáneas o sembradas, son un tipo de hábitat seminatural (Holland et al. 2016). Aunque estudios previos han demostrado que las cubiertas vegetales incrementan las poblaciones de crisópidos en el cultivo, estos estudios son realizados sobre mezclas de especies y no se identifica el papel funcional de cada especie en relación con los crisópidos (Wyss 1995, Porcel et al. 2017). Por ello, nuestro estudio (capítulo 4) se basa en la siembra individual de herbáceas de semillas nativas del área Mediterránea y todas las especies ensayadas se muestrearon de forma simultánea en el período de floración tanto en 2016 como 2017.

Se ensayaron 42 especies herbáceas (40 en 2016 y 22 en 2017), de las cuales 36 mostraron un desarrollo vegetativo adecuado, con un período de floración anterior al registrado en el olivo. De ellas 28 fueron empleadas por los crisópidos como lugar para reproducirse, alimentarse y/o descansar. Este estudio, reveló la existencia de un efecto

positivo entre la especie herbácea y las presas potenciales sobre la abundancia de crisópidos.

La comunidad de crisópidos colectada estuvo compuesta por *C. lucasina* y *C. pallida*, siendo la primera la especie dominante en número de individuos capturados. Entre las presas potenciales conocidas como atractivas para los crisópidos en estudios previos (Canard et al. 1984): ácaros, áfidos, colémbolos y trips, únicamente los trips mostraron una correlación positiva y significativa con la abundancia de crisópidos. Teniendo en cuenta los criterios entomológicos y agronómicos: atractividad para los crisópidos, disponibilidad de presas potenciales, desarrollo vegetativo de la planta y la incidencia indirecta de la planta sobre enfermedades que puedan afectar al olivo, las especies herbáceas más adecuadas para ser usadas para incrementar las poblaciones de crisópidos y en la restauración de cubiertas en el olivar serían: *Biscutella auriculata*, *Borago officinalis*, *Silene colorata*, *Crepis capillaris*, *Nigella damascena* y *Papaver rhoeas*.

8.3. Referencias

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Conclusiones

El análisis y discusión de los resultados en la presente tesis, ha generado las siguientes conclusiones:

1. La metodología y el esfuerzo de muestreo aplicado para la colecta de las distintas fases de desarrollo de la familia Chrysopidae han sido satisfactorias, puesto que han permitido obtener resultados estadísticamente significativos con respecto a los objetivos planteados.
2. El almendro, encina y pino ubicados en los hábitats seminaturales adyacentes al olivar presentan una idoneidad similar como lugares de oviposición para nueve especies de crisópidos. Las especies que realizan la puesta de forma aislada son las más abundantes, utilizando fundamentalmente el borde y haz de la hoja.
3. Parasitismo, depredación y mortalidad por factores desconocidos son los tres factores que inciden negativamente sobre las poblaciones de las fases juveniles de los crisópidos en las cuatro especies arbóreas estudiadas, siendo el parasitismo el más importante.
4. El olivo registró la mayor abundancia de fases juveniles colectadas con las menores tasas de parasitismo y el mayor número de juveniles que llegaron a completar su desarrollo hasta adulto. Todo ello convierte al olivo en la especie de árbol que proporciona una mayor contribución de crisópidos viables para la próxima generación al biotopo estudiado.
5. La diversidad de especies de crisópidos en fases juveniles que utilizan las cuatro especies arbóreas estudiadas es variable, siendo notable que *P. prasinus* está presente a lo largo del año en el olivo, la encina y el almendro, mientras que *C. mediterránea* está asociada casi exclusivamente con el pino durante la primavera y el verano.
6. El complejo parasitario de las fases juveniles se compone de cinco especies. *Baryscapus impeditus* es la especie de parasitoide dominante y está asociada a los juveniles de *Chrysoperla mediterranea*, *Chrysoperla lucasina* y *Chrysopa pallens*, las cuales se recolectaron sobre almendro y pino. *Helorus ruficornis*, el segundo parasitoide en importancia, está asociado a las especies de los géneros *Pseudomallada* (*P. picteti*, *P. flavifrons* y *P. prasinus*) y *Cunctochrysa* (*C. baetica*), que se colectaron preferentemente sobre la encina.
7. La riqueza de especies correspondiente a la fase adulta asociadas con cada especie de árbol fue diferente oscilando entre 11 y 14. Destaca la preferencia de *Pseudomallada*

prasinus por la encina, *Chrysoperla lucasina* por el almendro, *Chrysoperla mutata* y *Chrysoperla pallida* por el olivo, y *Chrysoperla mediterranea* por el pino.

8. La abundancia media de adultos y larvas de crisópidos capturados mediante succión en el olivo no se ha correlacionado con la depredación de huevos de *Prays oleae* en la generación antófaga y carpófaga.
9. La cobertura específica de las familias botánicas más abundantes en la cubierta y en la linde-mancha en los olivares estudiados no mostraron relación con las especies de crisópidos dominantes en fase adulta.
10. Numerosas especies herbáceas nativas del area mediterránea son utilizadas por *C. lucasina* como lugar de refugio, reproducción y/o alimentación. Es de destacar la atracción de *Biscutella auriculata*, *Borago officinalis*, *Silene colorata*, *Crepis capillaris*, *Nigella damascena* y *Papaver rhoeas* por este crisópido.