

TESIS DOCTORAL

Biología, interacciones y uso del depredador *Sphaerophoria rueppellii* (Diptera: Syrphidae) en el Control Integrado de plagas de áfidos en cultivos de invernadero

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de Alicante



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Tesis doctoral

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el Control Integrado de plagas de áfidos en
cultivos de invernadero**

Biology, interactions and use of predator *Sphaerophoria rueppellii*
(Diptera: Syrphidae) on Integrated Management of aphid pests in
greenhouse crops

Centro Iberoamericano de la Biodiversidad (CIBIO)
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el Control Integrado de plagas de áfidos en
cultivos de invernadero**

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optar al título de Doctor en Biología por la Universidad de Alicante

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Resumen

Los sírfidos afidófagos (Diptera: Syrphidae) son considerados agentes de gran potencial en el control biológico de pulgones, aunque a nivel comercial únicamente se encuentra disponible la especie *Episyrphus balteatus*. No obstante esta especie no es la más abundante, ni la mejor adaptada, a las zonas agrícolas del mediterráneo occidental. *Sphaerophoria rueppellii* es un sírfido afidófago presente de manera natural en el área mediterránea, abundante tanto en cultivos al aire libre como de invernadero. A pesar del interés de esta especie como posible agente de control biológico, poco se conoce acerca de su biología, siendo éste uno de los requisitos prioritarios en la selección de nuevos agentes de control. El objetivo general de esta tesis es aportar conocimiento sobre la potencialidad de este depredador en el control biológico de pulgones a través de dos grandes bloques temáticos. El primero se centra en el estudio de diferentes aspectos de la biología de *S. rueppellii*, las interacciones que establece con los áfidos que constituyen su presa y con otros agentes del mismo gremio con los que coincide espaciotemporalmente en los cultivos. En el segundo bloque experimental se evalúa la integración de este agente de control en dos estrategias englobadas en los planes de manejo integrado de plagas de pulgón en invernaderos. Los resultados obtenidos posibilitarán por una parte la cría masiva de esta especie, con vistas a su utilización en el control biológico, y aportarán el conocimiento necesario para su aplicación eficaz en estrategias de control integrado de plagas de pulgón.

En el **capítulo 1** “*Introducción general*” se expone la problemática actual en el control de plagas agrícolas, centrándose en el control biológico y en sus diversas estrategias. Posteriormente se presentan los pulgones y su importancia como insectos plaga, así como los sírfidos y los antecedentes sobre su papel como agentes de control de pulgones. Por último se revisan los aspectos que deben ser tenidos en cuenta para la selección de un nuevo agente de control biológico.

En el **capítulo 2** determinamos la influencia de factores abióticos como la humedad relativa y la temperatura en la mortalidad y tiempo de desarrollo de las fases preimaginales de *S. rueppellii*, así como en el tamaño de los adultos nacidos. Los resultados muestran que esta especie requiere elevadas humedades para completar su desarrollo, condición que pueden encontrar en determinados microhábitats de las plantas sobre las que se desarrollan, como el envés foliar o pliegues de las hojas. En cuanto a la temperatura, valores relativamente elevados permiten un desarrollo

adecuado de la especie y una menor duración de su ciclo biológico. Estos resultados confirman la idoneidad de este sírfido como agente de control biológico de pulgones en condiciones de humedad y temperatura elevadas, como las que se alcanzan en invernaderos mediterráneos, en las que el desarrollo de otras especies no es tan efectivo.

Además, en este capítulo también se ha evaluado la capacidad depredadora de la fase larvaria de *S. rueppellii*, y el efecto de la tasa de alimentación con el áfido *Myzus persicae* sobre el desarrollo y mortalidad de las distintas fases de su ciclo biológico, así como en el tamaño de los adultos nacidos. *Sphaerophoria rueppellii* se desarrolla adecuadamente en condiciones de escasez de alimento durante la fase larvaria, ya que es capaz de adaptar su tasa de consumo a la disponibilidad de pulgones. La mortalidad de huevo a adulto en estas condiciones no aumenta, aunque sí afecta al tiempo de desarrollo y al tamaño corporal de los adultos.

En el **capítulo 3** evaluamos el papel de los compuestos volátiles en la selección de lugares de oviposición por parte de las hembras de *S. rueppellii* y en el reconocimiento químico de potenciales competidores intragremiales tanto de naturaleza coespecífica como heteroespecífica. También estudiamos la importancia de los semioquímicos en la capacidad de búsqueda de colonias de pulgón durante la fase larvaria del sírfido. Los resultados mostraron que mientras que los volátiles emitidos por los áfidos no inducen ningún comportamiento significativo en las larvas de *S. rueppellii*, las hembras adultas ovipositan preferentemente en colonias de pulgón donde no hay competidores coespecíficos presentes. Nuestros ensayos también han demostrado que, en contra de lo esperado, los volátiles procedentes del coccinélido *Adalia bipunctata* no inducen una respuesta de evasión en las hembras de *S. rueppellii*.

En el **capítulo 4** evaluamos el efecto de la alimentación durante la fase adulta del sírfido con dos recursos florales frecuentemente utilizados en estrategias de manejo del hábitat; el mastuerzo marítimo (*Lobularia marítima*) y el cilantro (*Coriandrum sativum*) y con una especie cultivada (pimiento, *Capsicum annuum*) en la eficacia biológica de la generación parental y de la primera generación de *S. rueppellii*. Demostramos que la alimentación durante la fase adulta afecta a la eficacia biológica de la descendencia, y que las preferencias florales de los adultos están ligadas a una optimización del *fitness* de la siguiente generación. Cada especie de flor afectó a distintos componentes del *fitness* de *S. rueppellii*, evidenciando la importancia de

seleccionar las especies de flor más adecuadas para cada caso específico, a fin de asegurar los mejores resultados mediante esta estrategia de control por conservación.

En el **capítulo 5** estudiamos el efecto de las barreras fotoselectivas absorbentes de luz UV en el establecimiento de las sueltas de *S. rueppellii* en un cultivo hortícola y en su acción como agente de control sobre el pulgón *M. persicae* en esta situación de manejo integrado. En segundo lugar evaluamos la influencia de estas cubiertas en algunos componentes del *fitness* de *S. rueppellii* y en el comportamiento de búsqueda de recursos florales de los adultos. El resultado obtenido es que la actividad de los sírfidos adultos y de las fases inmaduras no está afectada negativamente de forma directa por el uso de estas mallas. La densidad de estadios inmaduros fue mayor, y su dispersión menor, bajo las mallas absorbentes de UV, lo que se asocia al efecto que estas mallas causan en el patrón de dispersión de los pulgones. Concluimos que la utilización de mallas fotoselectivas absorbentes de UV y las sueltas de sírfidos afidófagos como *S. rueppellii* son estrategias compatibles para el MIP de pulgón en invernaderos.

En el **capítulo 6** se discuten de manera global e integrada, los resultados obtenidos en los distintos capítulos y cómo la información aportada por este trabajo contribuirá a la futura utilización del sírfido *S. rueppellii* como nuevo agente de control biológico de pulgones.

Finalmente en el apartado dedicado a las **Conclusiones**, se resumen los principales resultados generados en los capítulos precedentes.

En el **Apéndice** de la tesis se citan otras publicaciones científicas y material científico-técnico elaborado a partir de los resultados de este trabajo. En segundo lugar, se listan las comunicaciones en congresos, seminarios y jornadas técnicas derivadas de los trabajos aquí incluidos. Por último, se recoge la labor de transferencia del conocimiento a la sociedad realizada durante el transcurso de esta tesis.

Abstract

Aphidophagous syrphids (Diptera: Syrphidae) are considered high-potential biocontrol agents of aphids, although there is only one commercially available species, *Episyrphus balteatus*. However, this is not the most abundant species, nor the best adapted, to the agricultural areas of the eastern Mediterranean. *Sphaerophoria rueppellii* is an aphidophagous syrphid that naturally occurs in the Mediterranean region, being abundant in both outdoor and indoor crops. In spite of the interest of this species as a biological control agent, little is known about its biology, and that is one of the priority requirements regarding the selection of a natural enemy for its use in augmentative biological control. The main purpose of the present thesis is to provide knowledge regarding the suitability of this predatory species on biological control of aphids through two main sections. The first one focuses on the study of different aspects of the biology of *S. rueppellii*, the interactions that it establishes with the aphids that serve as its prey and other biological control agents. The second section explores the consideration of this predatory species within other strategies involved in Integrated Pest Management of aphids in indoor crops. The study of these aspects will ease the mass rearing of this species, with the purpose of its application in biological and control of aphid pests. Furthermore, it will provide crucial information for its successful application within IMP strategies against aphids.

The **first chapter** is an introductory one and focuses on the current difficulties regarding control of agricultural pests, paying special attention to Biological Control and its diverse strategies. Secondly, it presents the aphids and their importance as pests, as well as syrphids and the previous knowledge regarding their role as biocontrol agents of aphids. Finally, the different aspects that are crucial before selecting a new biological control agent are examined.

In the **second chapter** we assessed the influence of relative humidity and temperature on mortality rate and developmental time of immature stages of *S. rueppellii*, as well as on the body size of emerged adults. Results show that this species requires high environmental humidity to properly develop. Such conditions may be found in microhabitats on the plants where they live, such the underside of leaves. Regarding the temperature, relatively high temperatures allow this species to fully develop and with a shortened developmental time. These results confirm the suitability of this syrphid as a biocontrol agent of aphids under high humidity and temperature

conditions, like those occurring in Mediterranean greenhouses, where other species are not as well adapted.

In this chapter we also evaluated the voracity of the larval stage of *S. rueppellii* and the effect of prey availability on developmental time and mortality rate of immature stages, as well as on adult body size. *Sphaerophoria rueppellii* is able to properly develop under low aphid availability conditions, since larvae are able to adapt their feeding rate to the food supply. Mortality rate from egg to adult did not increase under low food conditions, although developmental time increased, while adult body size decreased.

In the **third chapter**, we evaluated the importance of volatile compounds on the selection of oviposition spots by gravid females of *S. rueppellii*, as well as their role on the recognition process of potential conspecific and heterospecific competitors. Finally, we studied the importance of these compounds on searching behaviour of the larval stage of this syrphid. Results showed that volatiles from plants and aphids did not trigger a response on larvae, while female adults strongly preferred odours from aphid colonies without competitors. Our study also showed that while volatiles from conspecific individuals elicited a deterring response on *S. rueppellii* females, they did not react to those coming from coccinellid *Adalia bipunctata*.

In the **fourth chapter**, we assessed the influence of feeding on flowers of two insectary plants; sweet alyssum (*Lobularia maritima*) and coriander (*Coriandrum sativum*) and a crop species (sweet pepper, *Capsicum annum*) on fitness-related parameters of the parental and first generation of *S. rueppellii*. We found that adult nutrition affected the performance of the offspring, and that adult floral preferences were linked to an optimum performance of the next generation. Our study showed that each flower species had different effects on several fitness components of *S. rueppellii*, pointing out the importance of selecting the most suitable flower species on a case-specific basis for successfully implementing habitat management.

In the **fifth chapter** we studied the effect of UV-blocking photoselective films on the establishment and action of *S. rueppellii* as a biocontrol agent in a protected horticultural crop infested with aphid *M. persicae*. We also evaluated the effect of such covers on fitness-related parameters and foraging behaviour of adults. Results point out that the activity of neither adult nor larval stage is negatively affected by the use of UV-blocking films. Although syrphid immature density was higher, and dispersion lower, under UV-absorbing nets, the effect that these nets are known to have on the dispersion pattern of aphids may have indirectly caused these differences. We

conclude that the use of photosensitive nets and the release of syrphid predators such *S. rueppellii* are compatible strategies to be used in IPM aphid-control programs.

In the **sixth Chapter**, the results obtained in the previous chapters are discussed as a whole, and how these results will improve the knowledge and ease the future use of *S. rueppellii* as a biological control agent of aphids.

Finally, in the section devoted to the **Conclusions**, the main findings of the previous chapters are highlighted.

In the **Appendix** other scientific publications and technical documents derived from the results of this work are cited. Secondly, a list of all communications in congresses, seminars and working groups are listed. Finally, the work carried out regarding the transference of the knowledge to the society throughout this thesis is addressed.

Capítulo 1

Introducción general

1. Introducción

1.1. El Control Biológico

El control químico ha constituido una estrategia de gran importancia para el control de plagas desde que se comenzó a aplicar a gran escala en la década de los 50, permitiendo en su inicio un control eficiente tanto de las principales especies plaga como de las plagas de menor importancia económica (Ramakers, 2004). Sin embargo, en la actualidad existe una conciencia creciente sobre los múltiples problemas que su uso conlleva. Uno de ellos es el desarrollo, por parte de los organismos diana, de resistencias a los principales plaguicidas como consecuencia de su continua exposición a las materias activas, que ha reducido drásticamente su eficacia. El otro gran inconveniente es el perjuicio para el medio ambiente (toxicidad, contaminación, plagas secundarias, pérdida de biodiversidad, desaparición de la capa de ozono, etc.) (Anonymous, 1995) y para la salud humana. Como consecuencia, el número de materias activas de plaguicidas convencionales disponibles para los agricultores es cada vez más escaso, gracias a regulaciones tanto nacionales (BOE, 2012) como a nivel europeo (DOUE, 2009), donde cada vez se priorizan más los métodos de control no químicos y se establece un marco de actuación para conseguir un uso sostenible de los productos fitosanitarios que hoy se aplican con regularidad.

El **Control Biológico**, definido como “el uso de organismos vivos, de sus recursos, o de sus productos, con el fin de prevenir o reducir las pérdidas o daños causados por organismos plaga” (IOBC) es considerado actualmente como el método de control de plagas más seguro desde el punto de vista medioambiental, y también el más beneficioso económicamente (van Lenteren, 2011 y referencias incluidas). El riesgo de aparición de resistencias asociado a esta estrategia es muy bajo y su relación coste/beneficio es más favorable que para el control químico, incluso sin

considerar los costes derivados de los perjuicios ambientales de este último (Pimentel, 2009).

1.2. Control aumentativo y uso de especies nativas

Dentro del Control Biológico pueden diferenciarse una serie de estrategias, todas ellas encaminadas a reducir la densidad poblacional de las especies plaga: El control natural es la reducción de las plagas que tiene lugar en los ecosistemas naturales sin la intervención humana. El control clásico se basa en el uso de enemigos naturales alóctonos para controlar plagas que también son exóticas. El control por conservación se centra en proteger o estimular la acción de los enemigos naturales existentes en un entorno o región concreta mediante diversas acciones.

Otra de las estrategias comprendidas dentro del Control Biológico es el **control aumentativo**, que consiste en incrementar las poblaciones de enemigos naturales introduciendo en el cultivo individuos procedentes de crías masivas llevadas a cabo en biofactorías, a fin de obtener un control inmediato de las plagas (*control inundativo*) o de que se instalen en el cultivo, siendo las generaciones posteriores las que ejercen el control (*control inoculativo*) (Eilenberg et al., 2001). Esta práctica constituye una alternativa al control químico que es eficaz y segura desde el punto de vista medioambiental y económico, sobretodo en cultivos agrícolas y en particular en los cultivos de invernadero, donde las condiciones de manejo son más fácilmente controlables (van Lenteren and Manzaroli, 1999; van Lenteren and Bueno, 2003).

Los agentes de control usados en el control biológico aumentativo pueden ser especies *exóticas* (control biológico clásico) o *nativas*. A pesar de que el uso de especies introducidas puede ser efectivo a corto plazo, ya ha sido demostrado que puede derivar en problemas medioambientales, al interaccionar aquellas con el complejo de especies nativas de la zona. Un ejemplo es el caso reciente del

coccinélido *Harmonia axyridis* (Pallas), especie nativa del sudeste asiático, que desde su introducción en Europa en 1997 ha invadido ecosistemas naturales y actualmente es considerada como una amenaza potencial para los depredadores autóctonos (Pell et al., 2008). Otro de los motivos por los que la introducción de especies exóticas se perfila como ineficaz a largo plazo, es el hecho de que no están adaptadas a las condiciones ambientales o bióticas de cada zona, por lo que con el tiempo acaban perdiendo su efectividad. Es el caso del parasitoide de pulgón *Aphidius colemani* (Haliday) (Jacas et al., 2006), introducido en España en 1983, pero que actualmente cuenta con hiperparasitoides que han reducido considerablemente su eficacia. Por todos estos inconvenientes, la introducción de especies que no hayan pasado rigurosos protocolos de evaluación es inaceptable en la actualidad, habiendo surgido durante la última década una tendencia creciente a priorizar el uso de especies nativas de enemigos naturales. Este movimiento ya ha resultado en el reemplazo a nivel mundial de varias de las especies exóticas más populares por agentes autóctonos, libres de riesgos medioambientales y completamente adaptados a su área de actuación (van Lenteren and Bueno, 2003).

1.3. El problema de los pulgones en cultivos de invernadero Mediterráneos

La mayor parte de la producción Europea de hortícolas procede de la cuenca Mediterránea (FAOSTAT, 2009) y en concreto, esta región alberga la mayor superficie de cultivos hortícolas de invernadero a nivel mundial (Espí et al., 2006). Más del 20% de esta producción procede del sureste ibérico, donde la superficie destinada a producción orgánica e integrada bajo abrigo es cada vez mayor. Los cultivos protegidos de hortícolas, como el pimiento dulce (*Capsicum annuum* L.) (Solanaceae), presentan numerosas plagas de distinta incidencia económica, siendo las principales trips, mosca blanca, lepidópteros y pulgones (Pineda, 2008).

Los **pulgones** (Sternorrhyncha, Aphididae) son una de las plagas más importantes y dañinas que actualmente afectan a los cultivos de invernadero, especialmente a hortícolas (Ramakers, 2004). Además del daño directo que estos insectos ocasionan al alimentarse de la savia de floema, también causan graves daños indirectos al producir grandes cantidades de melaza, que queda sobre frutos y hojas propiciando el crecimiento de hongos, y al actuar como vectores de virus, que pueden tener efectos devastadores en la producción (Rabasse and Steenis, 2002; Blümel, 2004; Katis et al., 2007; Byers, 2008). Los casos más importantes que afectan a cultivos de invernadero a nivel mundial incluyen varias especies altamente polífagas, entre las que destacan *Aulacorthum solani* (Kaltenback), *Aphis gossypii* Glover, *Macrosiphum euphorbiae* (Thomas) y *Myzus persicae* (Sulzer) (Blackman and Eastop, 2000; Rabasse and Steenis, 2002; Blümel, 2004). No obstante, los esfuerzos para controlar estas plagas mediante estrategias de Control Biológico han sido limitados, debido a la disponibilidad de plaguicidas compatibles con los principales enemigos naturales, sumado a los dudosos resultados obtenidos con los parasitoides y depredadores empleados tradicionalmente, que han sido esencialmente los mismos durante más de dos décadas (Ramakers, 2004; Sánchez et al., 2011). Sin embargo, en los sistemas de producción integrada y ecológica, donde los tratamientos químicos están muy limitados, los pulgones son una de las plagas clave al carecer de una estrategia efectiva de Control Biológico. A esto se le suma además la elevada probabilidad de desarrollo de resistencias contra los escasos productos químicos permitidos. Por todas estas razones, existe una fuerte demanda de nuevas estrategias basadas en el Control Biológico que sean efectivas contra las plagas de pulgón en cultivos hortícolas de invernadero (Sánchez et al., 2011).

Varios trabajos han estudiado la ecología de parasitoides y pulgones asociados a los cultivos en España (e.g. Nebreda et al., 2005; Sánchez et al., 2011). En el segundo estudio, centrado en el cultivo de pimiento bajo abrigo en el sureste peninsular, se

observó que individuos de la especie *Aphidius matricariae* (Haliday), que entraban de forma natural en el invernadero, ejercían un mejor control sobre el pulgón *M. persicae* que las sueltas realizadas de la especie *A. colemani* que, no obstante, es la especie que continúa usándose casi en exclusiva para el control de este pulgón. Según van Lenteren (2011), existen todavía cientos o incluso miles de potenciales enemigos naturales por ser descubiertos, y el uso de los nuevos agentes que van descubriéndose se caracteriza por su alta tasa de éxito.

1.4. Los sírfidos en el control de plagas

La mayoría de las especies de artrópodos utilizadas en el control biológico aumentativo son himenópteros parasitoides (van Lenteren, 2011), tendencia que se hace extensiva al caso particular del control biológico de pulgones. La razón por la cual los parasitoides son los agentes de control mayoritarios es que en comparación con los depredadores, cuentan con una mayor especificidad, o lo que es lo mismo, un rango de hospedadores/presas más restringido, lo que se considera importante a la hora de seleccionar un enemigo natural (Bigler et al., 2006). No obstante, también existen depredadores de pulgón que cumplen este requisito.

Los **sírfidos afidófagos** (Diptera: Syrphidae) son considerados como agentes de gran potencial en el control biológico de los pulgones (Tenhumberg and Poehling, 1995; Rojo et al., 2003; Brewer and Elliott, 2004; Freier et al., 2007; Haenke et al., 2009). Las especies afidófagas representan el mayor porcentaje de esta familia (35%) y son también las mejor representadas en los agroecosistemas Mediterráneos. Aunque la mayor parte de los artículos sobre su importancia en el control biológico se centran en los cultivos de exterior (Niehoff and Poehling, 1995; Jansen, 2000; Miñarro et al., 2005; Aguado et al., 2006; Freier et al., 2007; Diaz et al., 2010), un número creciente de estudios muestran su relevancia también en cultivos de invernadero

(Pineda and Marcos-Garcia, 2008b; Pineda and Marcos-Garcia, 2008a; Pineda and Marcos-García, 2008b).

Los sírfidos afidófagos presentan 3 estadios larvarios antes de su pupación y todos ellos depredan sobre un amplio rango de especies de Homópteros de cuerpo blando (Rojo et al., 2003), entre las que se encuentran las implicadas en las principales plagas agrícolas. Las larvas se caracterizan por su voracidad, pudiendo consumir hasta 1000 pulgones durante esta fase de su desarrollo (Tenhumberg and Poehling, 1995; Rojo et al., 1996). Por su parte, la fase adulta es antófila, alimentándose de néctar y de polen (Schneider, 1969). Las hembras realizan la puesta cerca de colonias incipientes de pulgones, característica que unida a la movilidad del adulto, voracidad de sus larvas y larga permanencia en el cultivo por su polivoltismo, les convierte en agentes biológicos con potencial para controlar las poblaciones incipientes de pulgón, lo cual, debido al crecimiento exponencial de las mismas, es clave para el éxito de los planes de control biológico e integrado (Chandler, 1968b; Ankersmit et al., 1986; Chambers, 1988; Tenhumberg and Poehling, 1995).

A pesar de la probada eficacia de las poblaciones naturales de sírfidos afidófagos controlando las poblaciones de pulgón, a nivel comercial únicamente se encuentra disponible una especie, *Episyrphus balteatus* (De Geer), que pese a estar presente en nuestros ecosistemas, es particularmente abundante en el centro y norte de Europa. Sin embargo, esta especie no es la mejor adaptada a las zonas agrícolas del mediterráneo occidental (Pineda and Marcos-García, 2008b), presentando una elevada mortalidad a temperaturas por encima de 25°C (Hart et al., 1997) como las que se alcanzan frecuentemente en cultivos de invernadero del área mediterránea. La baja eficacia en el control conseguida con las sueltas de esta especie bajo las duras condiciones que se dan en los agrosistemas mediterráneos, ha propiciado que en la actualidad tanto las empresas productoras de insectos como los agricultores no presten a este grupo de depredadores la atención que se merece. Se hace necesaria

pues la búsqueda y selección de especies de sírfidos nativas de esta área, y por tanto adaptadas a sus condiciones ambientales y bióticas.

Sphaerophoria rueppellii (Wiedemann) es un sírfido afidófago de distribución Paleártica, pero es especialmente abundante en cultivos del área mediterránea (Speight, 2005). Esta especie ha resultado ser la mejor adaptada a los periodos de elevada temperatura y sequía que se dan en los agrosistemas mediterráneos, siendo además en estos periodos el principal representante del grupo en los cultivos protegidos del sureste peninsular (Pineda and Marcos-García, 2008b). Su gran potencial como agente para el Control Biológico de pulgones en cultivos de invernadero no ha pasado desapercibido, por lo que ha sido sugerida su comercialización y aplicación en el control de estas plagas (Pineda and Marcos-García, 2008b; Belliure et al., 2011). Sin embargo, para asegurar su éxito como nuevo agente de control biológico, se requiere ampliar el conocimiento sobre diferentes aspectos de la biología de esta especie, de los que todavía se conoce muy poco, y probar su compatibilidad con el uso de otros enemigos naturales y de otros medios de control.

1.5. La selección de un nuevo agente de control

Antes de poder usar de forma eficaz y segura un nuevo insecto como agente de control biológico de plagas, es necesario estudiar en profundidad una serie de aspectos que son imprescindibles para optimizar su manejo y para asegurar que se trata de un agente adecuado. Entre las primeras incógnitas que se deben despejar para conocer su capacidad de establecimiento y permanencia en el cultivo se encuentra el conocimiento, lo más completo posible, de sus requerimientos biológicos.

Es ampliamente conocido que **factores ambientales** como la temperatura y la humedad relativa influyen de manera importante en aspectos tales como la actividad,

desarrollo, reproducción y supervivencia de los insectos, condicionando de este modo su eficacia biológica (*fitness*) (Williams and Floyd, 1971). De igual modo, diferentes estudios han demostrado que las condiciones climáticas extremas que se alcanzan en el interior de los invernaderos afectan a la entrada, establecimiento, supervivencia y reproducción de los enemigos naturales (Shipp and Van Houten, 1997; Zhang and Shipp, 1998), influyendo negativamente en la eficacia de las especies que no están adaptadas a dichas condiciones. Por ejemplo, en sírfidos se ha visto que las bajas temperaturas alargan el periodo de pre-oviposición así como el tiempo de desarrollo, influyendo en el tiempo de generación y afectando a largo plazo a su eficacia controladora (Soleyman-Nezhadiyan and Laughlin, 1998). La **disponibilidad de presas** es otro factor que puede influir significativamente en el *fitness* de los insectos depredadores. Varios estudios han visto que algunas especies de sírfido son capaces de desarrollarse bajo condiciones de baja disponibilidad de pulgones, lo que puede responder a una adaptación a periodos de escasez de presas (Soleyman-Nezhadiyan and Laughlin, 1998). De igual modo, el conocimiento del número de presas que un depredador es capaz de consumir a lo largo de su vida es de especial importancia, pues la voracidad es uno de los factores que determinan cuán eficaz es un agente de control (Soleyman-Nezhadiyan and Laughlin, 1998).

Otra de las claves para el éxito de un nuevo agente de control biológico es el conocimiento de las relaciones que establece con los organismos fitófagos que constituyen su presas potenciales y con el complejo de enemigos naturales con los que coincide espacio-temporalmente en los cultivos (Wiedenmann and Wilson, 1996). Uno de los aspectos negativos de estas interacciones se da cuando el conjunto de especies que comparten una presa común (gremio) desarrollan una interacción trófica entre ellas, fenómeno conocido como **depredación intragremial**, que puede conducir al fracaso de los planes de control biológico (Polis and Holt, 1992; Rosenheim et al., 1995).

Los sírfidos son capaces de actuar como depredador intragremial al depredar sus larvas a áfidos ya parasitados (Meyhofer and Klug, 2002). Asimismo, otros trabajos han identificado una interacción negativa entre varias especies de sírfido y otros depredadores como coccinélidos o hemípteros, aunque la interacción suele ser asimétrica, siendo el sírfido la presa en la mayor parte de los encuentros (Hindayana et al., 2001; Frechette et al., 2007; Putra et al., 2009). A fin de minimizar estas interacciones antagonistas, los sírfidos han desarrollado una serie de estrategias comportamentales. Por ejemplo, se sabe que las hembras de *E. balteatus* son capaces de seleccionar colonias de áfidos libres de parasitoides para ovipositar, reconociendo y evitando los pulgones parasitados (Pineda et al., 2007; Almohamad et al., 2008). Asimismo, las hembras de algunas especies de sírfidos son capaces de adaptar su comportamiento de oviposición ante la presencia de depredadores coespecíficos (Almohamad et al., 2010b) o heteroespecíficos (Almohamad et al., 2010a). Reducir la competencia intra e interespecífica es crucial para este grupo, ya que sus estadios inmaduros son muy sensibles a la depredación y al canibalismo (Branquart et al., 1997; Hindayana et al., 2001; Frechette et al., 2007).

Junto a los estímulos visuales, gustativos y táctiles, en los últimos años se ha puesto de manifiesto el papel fundamental que los **compuestos volátiles** juegan en el proceso de atracción, reconocimiento y aceptación de los lugares de oviposición por parte de gran número de insectos, incluidos los sírfidos. Se sabe que la compleja mezcla de compuestos procedentes de la planta atacada por herbívoros (sinomonas), y la feromona de alarma de la presa (kairomonas), portan información sobre las características de la planta hospedadora, la especie de pulgón e incluso la edad de la colonia de áfidos que guían a las hembras en su búsqueda de los lugares de oviposición más adecuados (Dicke and Sabelis, 1988; Turlings et al., 1990; Turlings and Tumlinson, 1992; Vet and Dicke, 1992; De Moraes et al., 2001; Harmel et al., 2007; Verheggen et al., 2008). Por su parte, la percepción de compuestos químicos

procedentes de competidores de la misma o de distinta especie es capaz de actuar como estímulo disuasorio (Chandler, 1968a; Sadeghi and Gilbert, 2000; Almohamad et al., 2007; Almohamad et al., 2008; Verheggen et al., 2008; Almohamad et al., 2010a).

Así pues, el conocimiento de la comunicación química que se establece entre los tres niveles tróficos (planta-fitófago-enemigo natural) se presenta como una parte crucial para la comprensión de las interacciones entre los agentes de control que se desean liberar y/o los ya existentes, o entre la propia comunidad natural de enemigos naturales a fin de tomar las decisiones más adecuadas para asegurar el buen funcionamiento de los programas de control biológico. El hecho de que diferentes estudios arrojen resultados tan diferentes sobre el efecto y magnitud de las interacciones intragremiales en el control de plagas, indica la importancia del estudio de cada sistema particular.

1.6. Integración en otras estrategias de control

En el contexto de la **producción integrada**, donde las estrategias de control de plagas combinan varios métodos de control a fin de disminuir el daño que estas ocasionan, y con el menor perjuicio ambiental (Stern et al., 1959), el uso de enemigos naturales se complementa con otras estrategias tales como el control por conservación, o con medidas de control cultural, entre otras (Ehler, 2006). En este caso, la interacción entre el uso de los potenciales nuevos agentes y los distintos métodos de control también debe ser estudiado, a fin de seleccionar aquellas estrategias o combinación de las mismas que ocasionen el mínimo perjuicio a los organismos beneficiosos, o incluso que logren un efecto sinérgico. En palabras de Van Driesche and Bellows (1996) “es oportuno que la compatibilidad entre las distintas estrategias de control se evalúe desde la perspectiva del Control Biológico, puesto que

este se considera a menudo como el fundamento de todos los sistemas actuales de control de plagas”.

El **Control Biológico por conservación** se define como el manejo del agroecosistema con el fin de aumentar las condiciones favorables para el establecimiento y actividad óptimos de los enemigos naturales, o de minimizar los elementos que puedan influirles negativamente (Landis et al., 2000). Un ejemplo es la provisión de recursos que están ausentes o son insuficientes en el cultivo (**manejo del hábitat**), como hospedadores alternativos, hábitat refugio, o recursos tróficos, estrategias que ya han aportado resultados muy satisfactorios en sírfidos afidófagos, (Pineda and Marcos-García, 2008a; Pineda and Marcos-García, 2008b).

Una de las modalidades de manejo del hábitat especialmente eficaz para insectos dependientes de las flores como los sírfidos adultos, es la introducción de **recursos florales**. Se ha demostrado que la adición de plantas en flor alrededor y en el interior de los cultivos es capaz de aumentar el tiempo que estos insectos permanecen en los mismos, e incrementar significativamente su eficacia como agentes de control (Jervis et al., 1993; Al-Doghairi and Cranshaw, 1999), resultando en una reducción más efectiva de las poblaciones plaga, y un menor daño al cultivo (Hickman and Wratten, 1996; Pineda and Marcos-García, 2008a; Haenke et al., 2009). Además de su efecto atrayendo a los sírfidos y aumentando su permanencia en el cultivo, recientemente se ha visto que el uso de unas especies de flores u otras afecta a la eficacia biológica de los insectos, al influir en aspectos tan importantes como la fecundidad y la longevidad de las hembras (Scholz and Poehling, 2000; Laubertie et al., 2012). El estudio de los efectos que distintas especies de plantas con flor tienen en el *fitness* del agente de control biológico que se desea aplicar y en la consiguiente selección de las especies más adecuadas para cada caso, son aspectos de gran importancia a la hora de asegurar el éxito de los planes de control biológico e integrado.

El **control cultural** comprende una serie de estrategias alternativas al uso de fitosanitarios, que pueden contribuir a reducir la incidencia y evolución de las plagas. Por ejemplo, en el caso de cultivos de invernadero se ha demostrado que un adecuado manejo de la ventilación puede favorecer el control biológico, pues la apertura del invernadero en los momentos adecuados puede favorecer la entrada de enemigos naturales procedentes del exterior (Gabarra et al., 2004; Pineda and Marcos-García, 2008a).

Las **cubiertas fotoselectivas** constituyen otro ejemplo relativamente reciente, mediante el cual se bloquea o modifica la luz transmitida al interior de los invernaderos. Este cambio en el espectro de luz obtenido bajo estas cubiertas se ha aplicado con distintos propósitos, como modular el crecimiento de especies ornamentales, optimizar la producción y calidad del cultivo, o impedir la entrada y dispersión de insectos plaga al cultivo (Catalina et al., 2000; Díaz and Fereres, 2007 y referencias incluidas). En este último caso, las mallas fotoselectivas poseen una doble función: además de actuar como barreras físicas para la entrada de los insectos plaga a los invernaderos, son capaces de modificar el porcentaje de radiación ultravioleta del espectro solar que penetra en el cultivo, alterando de este modo la visión de los insectos. Varios estudios han demostrado que este “empobrecimiento” en luz UV altera el comportamiento de un gran número de especies plaga, resultando en una menor invasión de los cultivos, así como de una menor dispersión una vez dentro del cultivo (Chyzik et al., 2003; Legarrea et al., 2012a; Legarrea et al., 2012b).

También se ha probado que estas cubiertas pueden ejercer un efecto positivo en agentes de control biológico tales como hongos entomopatógenos, o algunos virus que actúan como insecticidas biológicos, aunque el número de estudios realizados sobre su efecto en los artrópodos que actúan como agentes de control, es todavía limitado (Díaz and Fereres, 2007). En el marco de la producción integrada, es crucial que ésta y otras estrategias de manejo sean compatibles con el control biológico a fin de

combinar ambas para conseguir un control eficiente de las plagas en cultivos de invernadero (van Lenteren and Noldus, 1990).

2. Objetivos

El objetivo general de esta tesis doctoral es el estudio de diferentes aspectos de la biología del sírfido depredador *Sphaerophoria rueppellii*, como sus principales requerimientos climáticos, las interacciones que establece con los áfidos que constituyen su presa y con otros agentes de control biológico, y su compatibilidad con otras estrategias que se utilizan frecuentemente en los planes de manejo integrado de plagas de pulgón en invernaderos. Para ello, se han desarrollado los siguientes objetivos específicos:

- **Requerimientos abióticos:** evaluar la influencia de la humedad relativa y temperatura ambiental en la eficacia biológica del sírfido.
- **Eficacia depredadora:** estudiar la voracidad de la fase larvaria y el efecto de la disponibilidad de presa en la eficacia biológica del sírfido.
- **Interacción intragremial:** evaluar la importancia de los compuestos volátiles de competidores intra- e interespecíficos en el comportamiento de oviposición de hembras del sírfido.
- **Manejo del hábitat:** estudiar la atracción de varios recursos florales y sus efectos en la eficacia biológica del sírfido. Seleccionar la especie o las especies de flor más adecuadas para este agente.
- **Control cultural:** valorar el efecto de las mallas fotoselectivas en la dinámica poblacional del sírfido, en su capacidad para localizar sus recursos alimenticios y en su eficacia biológica.

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Capítulo 2

Prey availability and abiotic requirements of immature stages of the aphid predator *Sphaerophoria rueppellii*

Disponibilidad de presa y requerimientos abióticos de las fases inmaduras del depredador de áfidos *Sphaerophoria rueppellii*

Amorós-Jiménez, R., Pineda, A., Fereres, A., Marcos-García, M. A., 2012. Prey availability and abiotic requirements of immature stages of the aphid predator *Sphaerophoria rueppellii*. *Biological Control* 63, 17-24.

Abstract

Aphidophagous hoverflies (Diptera: Syrphidae) are natural enemies with a high potential as biological control agents. Nevertheless, there is only one commercially available species, *Episyrphus balteatus*, which despite naturally occurring in the Mediterranean basin, is not the best adapted to the conditions found in its agricultural areas. *Sphaerophoria rueppellii* is another common hoverfly species in the Mediterranean area both in outdoor and greenhouse crops. However, little information about the biology of this species is known and this is one of the main requirements for every control-based IPM program. In this work we assess the influence of different humidity and temperature combinations on the performance (mortality, developmental time and sublethal effects) of *S. rueppellii* under controlled conditions. We also study the effect of aphid supply during larval stage on mortality and developmental time of immature stages and on sublethal effects on adults. The results show that this species requires high environmental humidity (higher than 60%) to complete its development. Also, *S. rueppellii* is able to properly develop at a wide range of temperatures (20-30°C). *Sphaerophoria rueppellii* reduces its voracity under low aphid density conditions, being able to adapt its feeding rate to the aphid availability. Mortality was not affected by food supply, while low prey availability led to longer developmental time and a decrease in larval and pupal weight and adult body size. These results show the suitability of this species as an aphid biological control agent under high humidity and temperature conditions, such as the ones inside Mediterranean greenhouses.

1. Introduction

The Mediterranean basin has the highest horticultural crop production of Europe (FAOSTAT, 2009), and the surface devoted to organic and integrated production increases every year, particularly in protected (i.e. greenhouse) crops (Freier and Boller, 2009). Horticultural crops have several key pests, aphids (Hemiptera: Aphididae) being one of the most important. More specifically, polyphagous species such as *Aulacorthum solani* (Kaltenback), *Aphis gossypii* (Glover), *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer) are the most damaging aphids in protected crops (Blümel, 2004; Rabasse and Steenis, 2002).

Several species of predators and parasitoids have been used in greenhouse crops against aphids, providing unsatisfactory results that led to the use of compatible chemicals to control this pest (Ramakers, 2004). In addition, biological control of aphids has been based on the same species for more than two decades, and only recently new and more-effective natural enemies have started to be commercialized and released for aphid control (Sanchez et al., 2011). Several studies have proven that the extreme climate conditions inside greenhouse vegetable crops can influence the establishment and reproduction of natural enemies (Shipp and Van Houten, 1997; Zhang and Shipp, 1998) as well as the development and survival of their immature stages, preventing most of the species traditionally used from a proper performance. For these reasons, there is a strong demand for effective biological control strategies against aphids on protected horticultural crops (Sanchez et al., 2011). Specifically, there is a current trend to encourage the search and use of indigenous natural enemy species, that has already led to the replacement of several popular exotic biological control agents with indigenous species (van Lenteren).

Aphidophagous syrphids (Diptera: Syrphidae) are high potential agents in aphid biological control (Brewer and Elliott, 2004; Freier et al., 2007; Haenke et al., 2009;

Tenhumberg and Poehling, 1995), being also compatible and complementary with the use of other biological control agents, such as parasitoid wasps (Pineda et al., 2007) or entomopathogenic fungi (Diaz et al., 2010). However, there is only one commercially available species, *Episyrphus balteatus* (De Geer). This species is particularly abundant and efficient on central and northern Europe (Tenhumberg, 1995), but it is not the most abundant in Mediterranean agricultural areas (Pineda and Marcos-Garcia, 2008b). A high mortality rate when temperature is higher than 25°C has been reported (Hart et al., 1997), a temperature condition which normally occurs in outdoor and indoor crops.

Sphaerophoria rueppellii (Wiedemann) is a predator hoverfly which usually occurs in Mediterranean crops (Pineda and Marcos-Garcia, 2008b; Speight, 2005). This species seems to be the best adapted to the extreme climatic conditions of high temperature and humidity, such as the ones occurring in Mediterranean areas, and particularly those taking place in greenhouses from southeastern Iberian Peninsula (Pineda and Marcos-Garcia, 2008b). In addition, previous work has shown that habitat management strategies such as providing additional floral resources (Pineda and Marcos-Garcia, 2008c) and banker plants (Pineda and Marcos-Garcia, 2008a) effectively enhance *S. rueppellii*'s populations in sweet-pepper Mediterranean greenhouses. Thus, this species has a high potential as an aphid biological control agent in protected crops, and it has been suggested to make this syrphid commercially available (Pineda and Marcos-Garcia, 2008b). However, little information about the biology of this species is known to date.

One of the first steps before natural enemies can be used effectively as mass reared biological control agents is to obtain valid information about their biology and their interaction with herbivore insects (Wiedemann and Wilson, 1996). This work aims to provide information about some biotic and abiotic requirements of the aphidophagous syrphid *S. rueppellii*, one of the main requirements previous to rear this

insect with commercial purposes. Our study system also consists of sweet-pepper [*Capsicum annuum* L. (Solanaceae)], a horticultural species largely extended in the Mediterranean area (FAOSTAT, 2009), and the aphid *M. persicae*, a species that causes severe economic losses in numerous crops worldwide (Blackman and Eastop, 2000; Minks and Harrewijn, 1989). We assess the influence of different humidity and temperature combinations as well as of food supply during larval stage, on the performance of immature *S. rueppellii* stages under controlled conditions as well as on sublethal effects on the developed adults.

2. Material and Methods

2.1. Plant material, aphids and syrphids

Sweet-pepper plants (*C. annuum* var. California Wonder) were grown from seed in a climate room ($T = 25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, $\text{RH} = 60\% \pm 10\%$, Photoperiod = 14L:10D, average light intensity = 495 lux) in plastic pots (5 x 5x 6 cm). These conditions were used throughout all of the rearing in this work. A stock colony of *M. persicae* was maintained on sweet-pepper plants in plastic boxes (30 x 30 x 60 cm) covered with a fine mesh on its upper part. The aphids were kept in a climate room with the same conditions as for plant growth. *Sphaerophoria rueppellii* cohorts were reared in plastic cages (30 x 30 x 60 cm), with a fine mesh on its upper part. The rearing cages contained bee granular pollen (© Sigma-Aldrich Co. LLC.), sugar provided as dry cubes, water (presented on a 100ml plastic glass inverted on a Petri dish lid with a disc of filter paper) and a sweet-pepper plant infested with the aphid *M. persicae* to stimulate oviposition. Larvae were reared on *M. persicae* colonies on sweet-pepper plants.

2.2. Abiotic requirements

To assess the effect of environmental conditions on immature stages of this hoverfly, three different temperatures (20°C, 25°C, 30°C \pm 1°C) and two relative humidity conditions (50%, 90% \pm 10%) were selected. Such conditions are common in both outdoor crops and greenhouses throughout spring and summer for a wide range of latitudes. Six treatments were established, representing all the possible combinations of those environmental conditions.

The temperature conditions needed were obtained by using three controlled FITOTRON cabinets, one for each temperature. Although this type of design mixes the effect of temperature and cabinet, it is a common problem among temperature cabinet studies and it is difficult to solve. The high humidity treatments were kept by using Petri dishes with a wet disk of paper inside and by sealing the dishes with Parafilm[®]. For low humidity treatments Petri dishes with a net on its upper part were used, so the humidity conditions inside the dish and that programmed on the rearing chamber were the same. Petri dish position was randomized and changed daily to compensate for any directional bias from the cabinet structure. Temperature and relative humidity inside the Petri dishes were checked by using Dataloggers (HOBO[®] U10-003). The Photoperiod throughout the experiment was 14:10 (L:D), and the average light intensity was 495 lux.

50 replicates were performed for each stage, consisting of a single egg, larva or pupa placed in a Petri dish (90 x 15 mm) with a piece of a sweet pepper leaf (1 cm²). Individuals from one assay were not re-used in other stage-assay. Eggs with the same age were obtained by placing an aphid-infested sweet-pepper plant in a rearing cage containing mature adults for two hours. Each egg was then removed from the plant by cutting off a small piece of plant tissue with the egg to avoid damage, and placed separately in a Petri dish. Mortality rate and time to egg hatching were recorded by

checking each replicate every 24h. Larval and egg mortality could be determined visually, whereas pupal mortality was scored when no adult emerged one week after the last adult emerged. A total of 50 eggs per treatment were observed.

Focusing on the larval stage, Ad libitum food supply (*M. persicae*) was provided throughout the larval cycle. Remaining aphids were removed daily and replaced with aphids from a rearing under controlled conditions, to avoid a negative effect in their quality due to temperature and humidity conditions inside the Petri dishes. The piece of sweet pepper leaf was removed daily as well, to make sure that the aphids remained on it without spreading on the dish. Larval mortality and developmental time of larvae were assessed by checking each Petri dish every 24h, from a total of 50 larvae per treatment.

Once larvae pupated, the pupae were kept under the conditions of the corresponding treatment. From the emerged adults, wing length was also determined, which is a good indicator of total body size in syrphids and other diptera and so, a good indicator of fitness (Stubbs and Falk, 1983). Specifically, the shortest length between the junction of the costal vein (C) with the humeral crossvein (h) and the junction of R4+5 with the medial vein (M) was measured (Dziock, 2005). Due to differences between males and females, measurements were analyzed separately for each sex. Morphological measurements were always done using the left wing, to avoid any effect due to asymmetry.

To study the effects on pupal stage, syrphid eggs and larvae were reared under the standard rearing conditions described above. Then each pupa was placed separately in a Petri dish, on a piece of leaf where the pupation occurred to avoid manipulation. Mortality rate and time of development were determined, as well as wing length of the emerged adults. A total of 50 pupae per treatment were observed.

2.3. Biotic requirements

To assess the effect of food supply during larval stage on the performance of this hoverfly, two treatments were established: The "high food availability" treatment, where a high number of aphids (*M. persicae*) were provided throughout the larval stage, and the "low food availability" treatment, where we provided larvae with a limited amount of aphids, enough to allow the larval stage to complete. The daily amount of aphids for each treatment (see Table 1) was based on a preliminary experiment where the maximum and minimum number of aphids consumed by *S. rueppellii*'s larvae were determined (see Supplementary Table 1). We provided larvae from "high food availability" treatment with aphids according to the maximum daily aphid consumption data, while "low food availability" treatment was supplied with aphids according to the minimum daily aphid consumption obtained in this preliminary trial.

Treatment/Day	1	2	3	4	5	6	7	8	9	10	11	12
Ad libitum	10	20	30	50	60	65	65	70	70	70	70	70
Limited food	1	5	5	15	25	25	35	35	35	35	35	35

Table 1: number of aphids provided daily on each treatment

Each replicate consisted of a one-day old larva placed separately on its own Petri dish with a piece of sweet pepper leaf (1 cm²), a wet disk of paper and sealed with Parafilm[®], to achieve 90% RH inside. Most of the aphids remained feeding on the leaf, so even first instar larvae, with a limited movement capacity (Chandler, 1968) were able to prey on the aphids that they needed. 60 replicates were performed for each treatment.

Every 24 hours remaining aphids in the Petri dish were removed and counted. After that, new aphids from a rearing were provided. Only third and fourth instar-aphids were provided, to avoid underestimating the predation owing to the potential progeny

production by aphid adults. Through observations every 24h, daily and total aphid consumption rate throughout the larval stage were determined, as well as mortality rate and time of development of both larvae and pupae. Also, the third instar larvae and newly formed pupae were weighed using a precision weighing scale (Acculab ALC-110.4 Analytical Balance). Pupae were placed in two 30 x 30 x 60 cm plastic rearing cages according to the treatment, with a fine mesh on its upper part. Emergence rate and wing length of adults were measured. Experiments were carried out with the same environmental conditions explained above.

Day	1	2	3	4	5	6	7	8	9	10	11
Mean ±	4.9 ±	8.7 ±	17.6	27.2	36.7	43.7	53.7	36.2	17.3	7.7 ±	
SE	0.6	0.9	± 1.4	± 1.8	± 1.9	± 2.4	± 1.5	± 5.9	± 4.8	3.8	
Minimum	1	4	5	16	25	24	38	1	3	2	15
Maximum	8	18	28	47	60	62	62	69	55	15	15

Supplementary Table 1. Daily aphid consumption by *S. rueppellii*. A total of 18 larvae (only consumption of larvae that survived is shown) of *S. rueppellii* were observed daily in individual Petri dishes, from egg hatch till pupation. Data represent the consumption of third- and fourth-instar *M. persicae* aphids, calculated as the difference between provided (20-100 aphids/day) and remaining aphids. Other applied methodologies are similar to the methods described in the manuscript.

2.4. Statistical analysis

The normality of the data was tested with the Kolgomorov-Smirnov and Shapiro-Wilk tests. Data on mortality rate, developmental time and aphid consumption were not normally distributed and therefore were analysed with Generalized Linear Models (GENLIN) followed by LSD post-hoc test for multiple pair-wise comparisons. The model was adjusted to a Poisson distribution with log link function for developmental time and aphid consumption, and to a binomial distribution with logit link function for mortality. Data on aphid consumption was analyzed with GENLIN for repeated measures (days). Data on wing length and weight were normally distributed and therefore were analyzed with a t-test or ANOVA, this followed by Tukey post-hoc test for multiple pair-wise

comparisons. Wing length data from the larvae experiment were analysed with one-way ANOVA test to study the differences among temperatures, due to the fact that survival of larvae was 0% in the 50%RH treatments. Data on wing length from the pupae experiment were evaluated with two-way ANOVA test to determine effects of relative humidity and temperature. All data were analysed with the statistical package SPSS V19.0.

3. Results

3.1. Abiotic requirements

Low humidity had a strong negative impact on the performance of *S. rueppellii*. Eggs (Fig. 1) and larvae (Fig. 2) were the most sensitive stages, with a higher mortality (egg: $df = 1, 299$; deviance ratio = 97.58; $P < 0.001$; larva: $df = 1, 299$; deviance ratio = 781.79; $P < 0.001$) and longer developmental time (egg: $df = 1, 151$; deviance ratio = 203.76; $P < 0.001$) when developing at 50%RH compared to 90%RH. Actually, larval mortality was 100% for all treatments when developing at low humidity, which made impossible to obtain data of their developmental time as well as pupa and adult parameters for these treatments. Interestingly, pupae were not affected by low humidity conditions ($df = 1, 299$; deviance ratio = 2.26; $P > 0.05$; Fig. 3).

The increase of temperature had no effect on egg and larva mortality (egg: $df = 2, 299$; deviance ratio = 2.13; $P = 0.121$; larvae: $df = 2, 299$; deviance ratio = 3.02; $P = 0.051$), whereas at 30 °C pupal mortality significantly increased compared with the other temperatures ($df = 2, 299$; deviance ratio = 5.81; $P = 0.003$; LSD: 20-30°C $P = 0.003$, 25-30°C $P = 0.02$). Interestingly, when larvae developed at 25°C or 30°C, the following pupal stage also had significantly higher mortality compared with larvae that

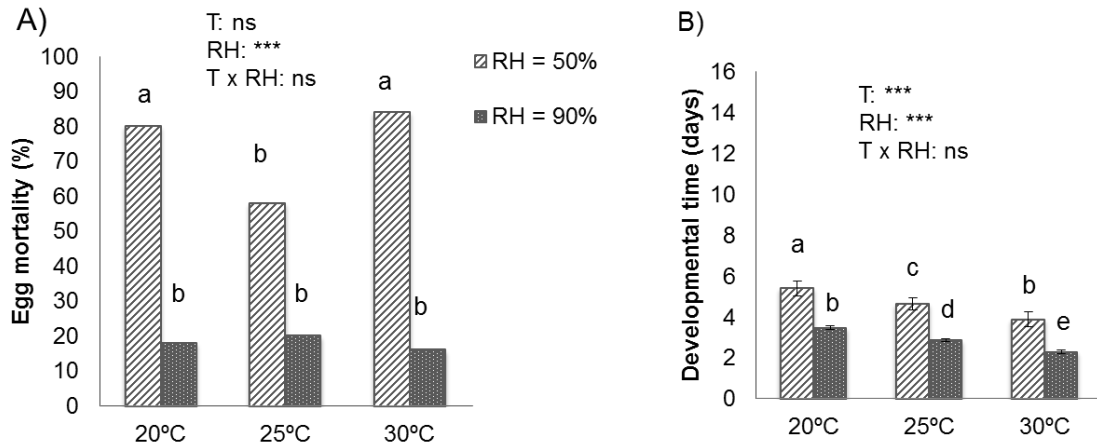


Figure 1: Abiotic requirements for eggs. A) Mortality rate (mean %). B) Developmental time (mean + SEM). Different letters above bars indicate significant differences (GENLIN, LSD).

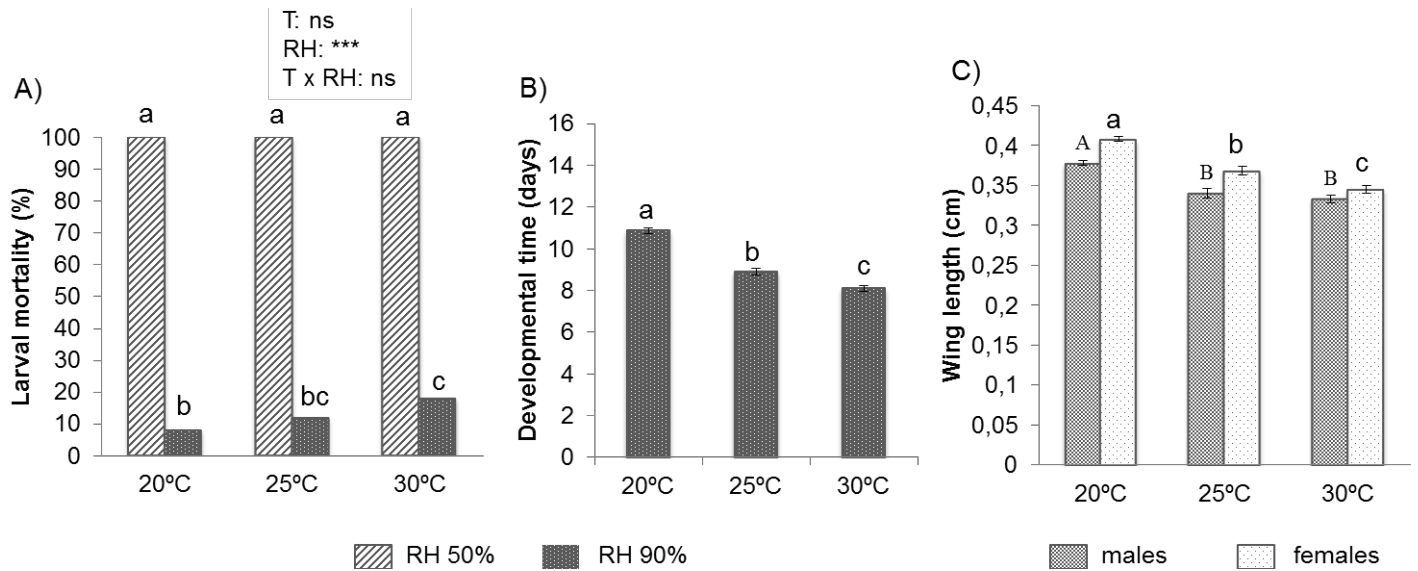


Figure 2: Abiotic requirements for larvae. A) Mortality rate (mean %). B) Developmental time (mean + SEM). C) Wing length of adults (mean + SEM). Different letters above bars indicate significant differences (GENLIN, LSD for mortality and developmental time, ANOVA and Post Hoc test with Tukey HSD for wing length).

developed at 20°C (df = 2, 149; Wald statistic = 7.213; $P = 0.03$; LSD: 20-25°C $P = 0.026$; 20-30°C $P = 0.008$) (see Supplementary Fig. 1). In contrast, temperature had a positive effect on the developmental time of the three *S. rueppellii* stages, which showed a faster development as the temperature raised (egg: df = 1, 151; deviance ratio = 203.76; $P < 0.001$; larvae: df = 2, 130; Wald statistic = 194.5; $P < 0.001$; pupae:

df = 2, 276; deviance ratio = 1293.58; $P < 0.001$; LSD, $P < 0.001$). However, the faster larval development had a negative consequence for the body size of the emerging adults, since the increase of temperature reduced wing length of males ($F_{2, 20} = 31.46$; $P < 0.001$) and females ($F_{2, 22} = 31.01$; $P < 0.001$ between 20°C and 25-30°C, $P < 0.05$ between 25°C and 30°C). Similarly, faster pupal development led to shorter wing length in both males (df = 2, 89; $F = 44.85$; $P < 0.001$) and females (df = 2, 89; $F = 48.48$; $P < 0.001$) suggesting that this reduction in body size is independent of a lower food intake.

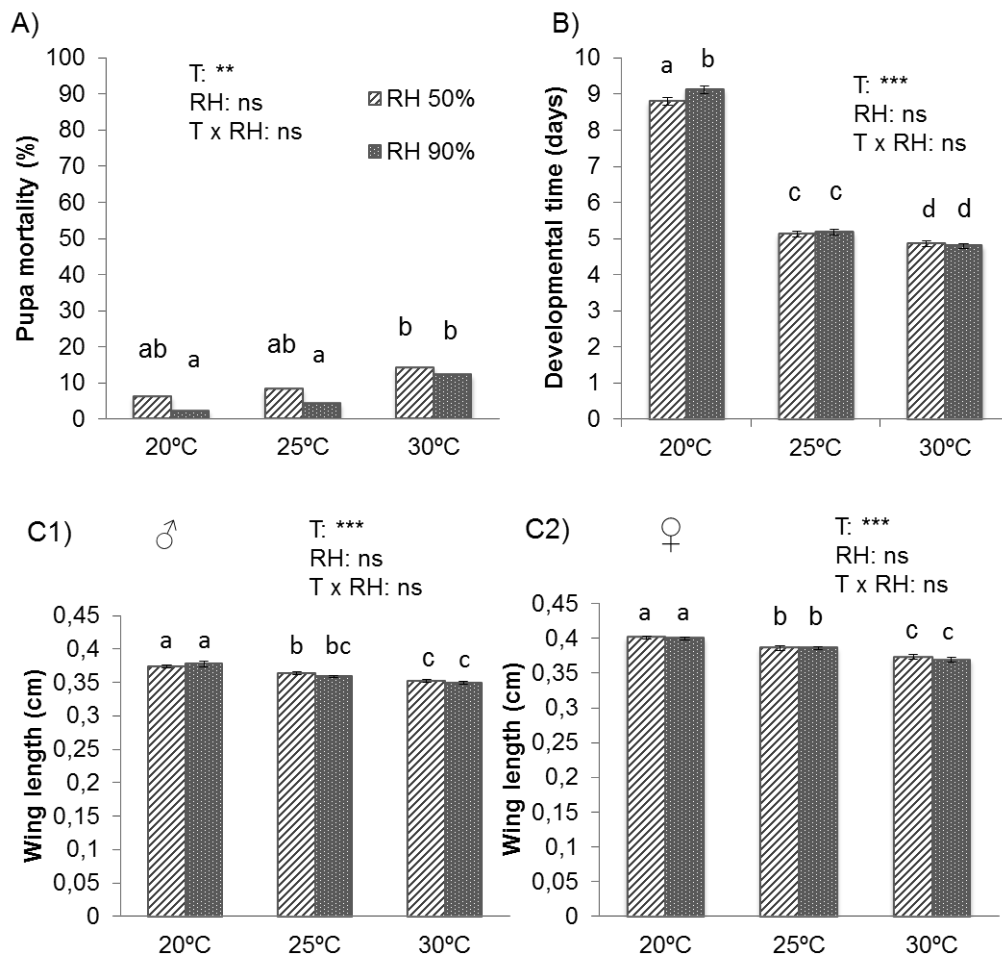


Figure 3: Abiotic requirements for pupae. A) Mortality rate (mean %). B) Developmental time (mean + SEM), C) Wing length of males and females (mean + SEM). Different letters above bars indicate significant differences (GENLIN, LSD for mortality and developmental time, two-way ANOVA and Post Hoc test with Tukey HSD for wing length).

3.2. Biotic requirements

The daily feeding rate of larvae (Fig. 4) was affected both by the level of food supply (df = 1, 1115; Wald statistic = 21.5; $P < 0.001$) and by the day of development (df = 11, 1115; Wald statistic = 7618.8; $P < 0.001$). The maximum aphid consumption was achieved 2 days later on the low food availability treatment, and the developmental time was also 2 days longer in this treatment (Fig. 4). A limited supply of aphids, had no impact on larval (df = 1, 89; Wald statistic = 0.0001; $P = 0.992$) nor pupal (df = 1, 67; Wald statistic = 1.323; $P = 0.254$) mortality of *S. rueppellii* (Fig. 5A). However, limited food supply increased the developmental time of larvae (Fig. 5B) compared with the high food availability treatment (df = 1, 92; Wald statistic = 140.7; $P < 0.001$), whereas pupal developmental time remained unaffected (df = 1, 86; Wald statistic = 0.192; $P < 0.663$). As we expected, limited food supply had a negative effect on the weight (Fig. 5C) of third instar larvae (df = 53, $t = 4.71$, $P < 0.001$), and newly formed pupae (df = 68, $t = 4.81$, $P < 0.001$). In the same way, the wing length of both adult females (df = 17, $t = 2.49$, $P = 0.023$) and males (df = 15, $t = 3.39$, $P = 0.004$) decreased with limited food supply during larval stage (Fig. 5D).

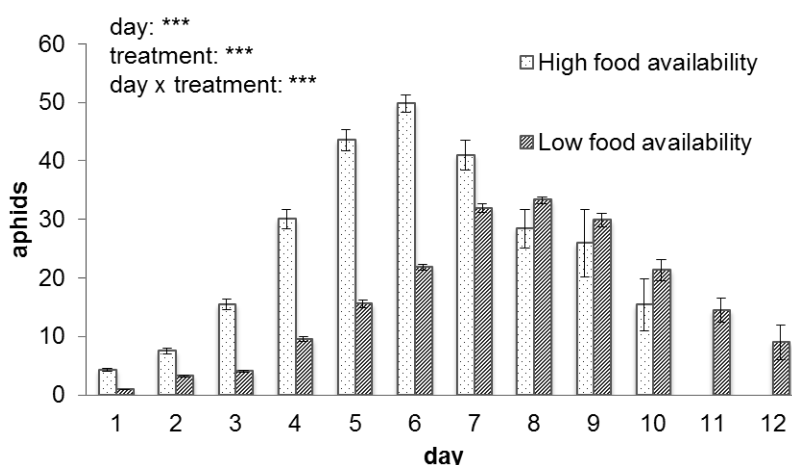


Figure 4: Daily aphid consumption rate (mean + SEM) throughout larval stage on high and low food availability treatments. Data were analyzed with a GENLIN for repeated measures.

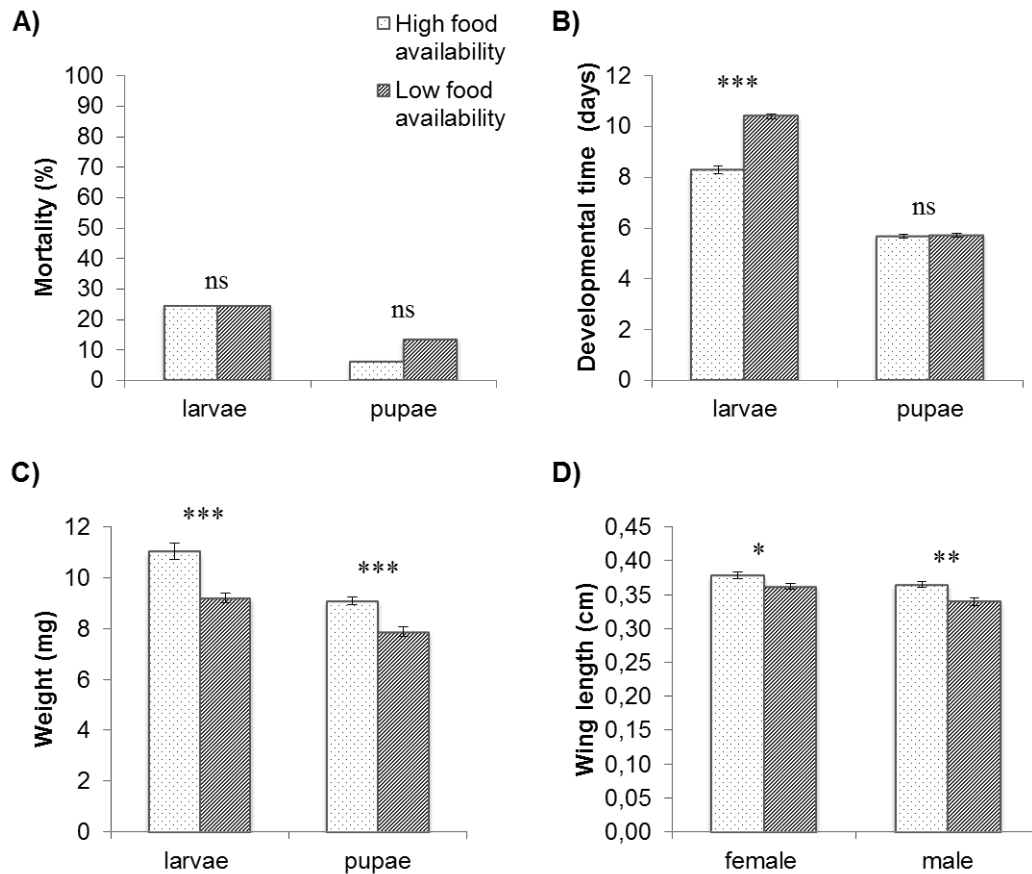


Figure 5: Performance with different aphid supply. A) Developmental time of larvae and pupae (mean + SEM). B) Mortality rate of larvae and pupae (mean %). C) Weight of larvae and pupae (mean + SEM). D) Wing length of adults (mean + SEM). ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (GENLIN for developmental time and mortality, T-test for independent samples for body weight and wing length).

4. Discussion

4.1. Abiotic requirements

The results of this study show that abiotic conditions have a key role on the development of different immature stages of *S. rueppellii*. Pineda and Marcos-García (2008b) evaluated the composition and population dynamics of aphidophagous syrphids inside sweet-pepper greenhouses from southeastern Spain between 2004 and 2006. They found out that *S. rueppellii* was much more abundant than the rest of species in the years when drought periods happened earlier and lasted longer,

suggesting that this species was the best adapted to the dry conditions occurring in Mediterranean areas compared with the other syrphid species identified, including *E. balteatus* and *Eupeodes corollae* (Fabricius). Additionally, such study showed that *S. rueppellii* appeared later in the season than the other syrphid species, when temperatures were higher. Based on that study, we expected that the performance of *S. rueppellii* would improve at high temperature and low humidity. In contrast to our initial expectations, our results do not support the hypothesis that *S. rueppellii* develops better under dry conditions. Specifically, this study shows that egg and larval stages are particularly susceptible to low relative humidity, whereas pupal stages are not affected by the humidity conditions (measured as developmental period, mortality rate and adult's wing length). However, the potential of this species to naturally establish on protected crops and efficiently control aphid populations would be favoured by the high humidity conditions occurring inside greenhouses.

Hoverflies may have several strategies to tolerate drought conditions in the field, such as the use of microhabitats or the adjustment of their activity schedules. Syrphid larvae are generally more active during the night than in daylight (Holmes, 1985; Vickerman and Sunderland, 1975), although we have observed in the field that *S. rueppellii* has also a voracious predatory activity during the day. On the abaxial (lower) surface of leaves, which cannot be directly reached by light, a higher relative humidity and lower temperature occurs compared with the adaxial (upper) surface because of the higher stomata density (Gutschick, 1999). Personal observations both in laboratory and field conditions indicate that *S. rueppellii*'s females lay their eggs preferably on the reverse of leaves and larvae can normally be found here and on other areas of the plant where the humidity has been condensed. Regarding the pupal stage, almost all the pupation observed in controlled conditions takes place in plant areas with a high condensation, such as the back of the leaves, places where a leaf is on top of the other, dead leaves or roots and fibres present on the substrate. These observations

suggest that under low humidity conditions, like the ones occurring in outdoor crops or outside greenhouses, *S. rueppellii* could exploit these microhabitats where the optimal humidity and temperature conditions for its development and activity may be reached.

Pupa is the only stage for which mortality is affected by temperature independently of the relative humidity, increasing when the pupa is exposed to higher temperatures. This indicates that, although pupal developmental time and mortality are not affected by relative humidity, this immature stage is the most sensitive to the surrounding temperature. Development of all immature stages was completed faster when the temperature rose, with the shortest developmental period taking place at 30°C. Honek and Kocourek (1988) and Saidov (1974) found this negative relation between temperature and developmental period in more than 20 species of aphidophagous insects, including syrphids such as *E. balteatus* and *Sphaerophoria scripta* (L.).

The relation between body size, fitness and temperature has been documented on a high number of animals, especially on insects (Atkinson, 1994), and it can be summarised in two rules. The first rule '*hotter is smaller*' proposes that ectotherms that develop at higher temperatures will be relatively small as adults (Angilletta and Dunham, 2003; Atkinson, 1994), and our results on wing length support this pattern. The second rule '*Bigger is better*' proposes that individuals with larger body size will tend to have greater performance and fitness than smaller individuals (Bonner, 2006; Peters, 1983) in the sense of a greater survival, fecundity and mating success (Kingsolver and Pfennig, 2004). According to this rule, our results of wing length suggest a negative effect of the temperature increase on *S. rueppellii* performance, as the mortality results also show. Nevertheless, some authors (Frazier et al., 2006; Savage et al., 2004) suggest that there is another rule (*Hotter is better*), proposing that species that have adapted to high optimal temperature can also have enhanced fitness at high temperatures, through a shortened generation time (Roff, 2002). Interestingly, our results also support this hypothesis, since the three larval stages developed faster

with increasing temperatures. Since *S. rueppellii* provides a case of a species that has evolutionarily adapted to warm climate, it will be an excellent candidate to evaluate whether the benefit of shorter developmental time caused by high temperatures overrides the fitness cost of the decrease in body size.

Immature stages of *E. balteatus*, the only syrphid species in commercial production, suffer a high mortality with temperatures over 25°C (Hart et al., 1997). In previous studies, we monitored the temperature in several commercial greenhouses of southeastern Spain during two years, and temperatures in spring varied between 11°C (during night) and 38°C (in daytime). We believe that *S. rueppellii* is a high potential control agent on Mediterranean greenhouses, due to a better tolerance to the high temperatures present on these environments. Despite there are evidences that *S. rueppellii*'s performance decreases when developing at high temperatures, this is the most abundant species in Mediterranean greenhouses towards the end of spring, when the daily maximum temperature is over 25°C, indicating that out of the species recorded, this hoverfly is the best adapted to the high temperature conditions present inside greenhouses (Pineda and Marcos-Garcia, 2008b). Augmentative biological control is an important part of IPM programs, and the availability of several natural enemies that are effective under different abiotic conditions will contribute to the success of pest control under a changing climate. In addition, the use of native species and the high specificity of aphidophagous hoverflies ensures a lack of side effects to the environment and negative intraguild interactions with other natural enemies, which is another key point for satisfactory results of biological control strategies.

4.2. Biotic requirements

Ovipositional preference by females has a profound effect on the performance of their offspring, ensuring the newly emerged larvae have enough food to develop

(Sadegui and Gilbert, 2000). However, some indications imply that even small syrphid larvae must move between plants in search for new aphid colonies (Banks, 1968) as a single aphid colony may be insufficient to support larval development to maturity (Kan, 1988a; Kan, 1988b). *Sphaerophoria rueppellii* is able to complete its life cycle even under low aphid availability conditions, with survival of larvae not being affected by diet. This fact could allow the larvae to exploit small and developing aphid colonies, moving among different plants or areas within a plant, which is a very desirable feature on a biological control agent. Several authors state that aphid quantity and quality is a major factor influencing the development time of aphidophagous syrphids (Dziocik, 2005; Polak, 1980; Rojo et al., 1996). Limited food supply increases the developmental time of *S. rueppellii*'s larvae while, interestingly, the developmental period of pupal stage does not change in accordance with aphid's consumption throughout larval stage. This trend has also been observed on the hoverfly *E. corollae*, while in other species such as *E. balteatus*, pupal development shortens when larvae are provided with excess of aphids (Rojo et al., 1996). Nevertheless, a reduced weight of immature stages and wing length of adults is reached when aphid supply is not high enough. Consequently, larvae's diet has a major effect on body size of *S. rueppellii*, like has been reported on other hoverfly species like *E. corollae* and *E. balteatus* (Rojo et al., 1996). Body size is correlated with a large number of species' attributes that influence their fitness, including longevity, reproductive rate or resource use (Blackburn and Gaston, 1994; Harvey and Pagel, 1991).

Daily feeding rate under high food availability conditions reaches a peak once larvae have reached the third instar. Adams et al. (1987) and Soleyman-Nezhadiyan and Laughlin (1998) found that the last larval instar of syrphids is also the most voracious, been responsible of up to 90% of the total aphid consumption. Mean daily consumption under high food availability conditions was $26,11 \pm 4,89$ aphids/day, a slightly lower value than the one measured by Ming Hong and Quang Hung (2010) with

the species *E. balteatus* feeding on *M. persicae* ($31,4 \pm 1,88$ aphids/day). Under low food availability conditions, the maximum aphid consumption of *S. rueppellii*'s larvae is postponed two days, since third instar is reached later than in optimal food conditions. Our results agree with what has been exposed by several authors, which assure that the proportion of prey consumption during the first few days after eclosion is small (Adams et al., 1987; Barlow, 1979), and it decreases again towards the end of larval stage (Tenhumberg and Poehling, 1995).

Although the developmental period of the larvae is two days longer with limited aphid supply, the total aphid consumption is higher under high food availability conditions, being able to prey on nearly 220 aphids throughout this stage, a higher value than the one found by Hopper et al. (2011) with a species from the same genera, *Sphaerophoria sulphuripes* (Thomson) with a lifetime consumption of 194.44 third instar aphids. Even though aphid supply on the Lowfood availability treatment was below the optimal, *S. rueppellii*'s larvae hardly consumed all the aphids provided. The average number of non-preyed aphids that remained on the Petri dishes was 7.61 individuals (30% of the aphids supplied). A possible explanation to this may be that at very low aphid densities *S. rueppellii*'s larvae could be able to adapt their feeding rate according to the food availability conditions, as found by Tenhumberg and Poehling (1995) on some syrphids such as *E. balteatus* and *S. scripta*.

5. Conclusions

Because aphid populations grow exponentially, biological control strategies by using hoverflies should achieve a greater impact on aphid population during an early stage of the infestation (Tenhumberg and Poehling, 1995). Under low aphid density conditions, sublethal effects are observed on the *S. rueppellii* adults developed in such conditions, highlighting a reduced fitness in such conditions. According to this, the use

of inoculative releases would not be effective, because under persistent low food availability conditions, the number of syrphids might not be sufficient to sustain a permanent population in the field and new releases would be needed in order to keep controlling the pest population. On the other hand, the fact that mortality rate of larvae does not increase even under low aphid density conditions, supports the potential use of this predator on augmentative control strategies through inundative releases. In addition, the fact that this species is adapted to the high humidity and temperature conditions similar to those occurring in Mediterranean greenhouses makes this species even more suitable for its use in these environments. However, estimating predatory efficiency of syrphid larvae based on laboratory estimates may lead to an overestimation on their actual impact on the pest in the field (Tenhumberg and Poehling, 1995). Additional factors may affect the efficacy and population dynamics of predators in field conditions, such as intraguild predation (Rosenheim et al., 1995), variation of environmental conditions and prey quality (Symondson et al., 2002), sublethal effects of pesticides (Desneux et al., 2007) or habitat management strategies (Landis et al., 2000), pointing out that field studies are needed in order to evaluate *S. rueppellii*'s effectiveness as an aphid control agent in commercial greenhouses.

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Capítulo 3

A differential role of volatiles from conspecific and heterospecific competitors in the selection of oviposition sites by the aphidophagous hoverfly *Sphaerophoria rueppellii*

Efecto diferencial de los volátiles de competidores coespecíficos y heteroespecíficos en la selección de los lugares de oviposición en el sírfido afidófago *Sphaerophoria rueppellii*

Amorós-Jiménez, R., Robert, Marcos-García, M. A., Fereres, A. & Turlings, T. C. J. 2013. A differential role of volatiles from conspecific and heterospecific competitors in the selection of oviposition sites by the aphidophagous hoverfly *Sphaerophoria rueppellii*. Submitted to Animal Behaviour.

Abstract

It is known that the selection of oviposition sites by syrphids and other aphidophagous insects is influenced by the presence of conspecific and heterospecific competitors. Chemical cues play an important role in the recognition process, some of them being volatile compounds. Yet, little is known about the identity and specificity of semiochemicals that are involved in the foraging behaviour of these predators. In this study, we used olfactometer bioassays to explore the olfactory responses of gravid females and larvae of the syrphid *Sphaerophoria rueppellii*, focussing on volatiles from conspecifics, as well as the competing coccinellid *Adalia bipunctata*. In addition, a multiple-choice oviposition experiment was conducted to study if females respond differently when they can also perceive their competitors through visual and tactile cues. Results showed that volatiles from plants and aphids did not affect the behaviour of second-instar larvae, whereas adult females strongly preferred odours from aphid colonies without competitors. A repellent effect was only evident for the odour of conspecific *S. rueppellii* individuals, whereas the choices by adult females were not affected by volatiles coming from heterospecific *A. bipunctata*. Low incidence of coexistence of both predators related to their differential occurrence in Mediterranean agricultural areas might explain this absence of a response to the intraguild competitor.

1. Introduction

Syrphid larvae are important natural enemies of aphids (Brewer and Elliott, 2004; Freier et al., 2007; Haenke et al., 2009; Rojo et al., 2003; Tenhumberg and Poehling, 1995). Together with generalist coccinellid predators they represent the most important natural agents that reduce the population densities of aphids in agricultural ecosystems (Coderre, 1999; Freier et al., 2007; Latham and Mills, 2009; Smith et al., 2008; Winder et al., 1994).

Ovipositional preferences by syrphid females has a profound effect on the performance of their offspring, ensuring that newly emerged larvae have enough food for full development (Sadegui and Gilbert, 2000) and limiting their exposure to cannibalism and intraguild predation (Branquart et al., 1997; Frechette et al., 2007; Hindayana et al., 2001). Therefore the selection of oviposition sites must reflect a preference of high quality aphid patches, with the 'quality' depending not only on the presence and density of aphids, but also on the presence of inter-and intraspecific competitors (Almohamad et al., 2010b; Pineda et al., 2007). Oviposition site selection may rely greatly on the perception of the prey-derived volatiles and herbivore-induced plant volatiles (synomones) (De Moraes et al., 2001; Dicke and Sabelis, 1988; Harmel et al., 2007; Turlings and Tumlinson, 1992; Turlings et al., 1990; Verheggen et al., 2008; Vet and Dicke, 1992). Such volatiles provide syrphid females with information about host plant characteristics, aphid species and even the presence of conspecific and heterospecific competitors (Almohamad et al., 2008b; Almohamad et al., 2007; Almohamad et al., 2010a; Chandler, 1968; Sadeghi and Gilbert, 2000; Verheggen et al., 2008).

Because larvae of aphidophagous hoverflies and ladybird beetles occur frequently on the same aphid infested plants, these predators may strongly interact with each other, especially when their prey becomes scarce (Agarwala and Yasuda, 2001).

Previous studies have assessed the oviposition-avoidance behaviour from syrphid females to chemical cues from conspecific and heterospecific (e. g. coccinellid) competitors (Almohamad et al., 2010a; Almohamad et al., 2010b; Hemptinne and Dixon, 2000; Laubertie et al., 2006; Verheggen et al., 2008). However, these past studies did not distinguish between the role of volatile compounds and both gustatory and visual stimuli. These latter factors are also important for aphid colony selection by syrphid females (Chandler, 1966). The present work aimed to study, to our knowledge for the first time, the specific role of volatile compounds on the interaction between syrphids and conspecific and heterospecific competitors.

We used a system consisting of sweet-pepper [*Capsicum anuum* L. (Solanaceae)], a horticultural species largely extended in the Mediterranean area, and the aphid *Myzus persicae* (Sulzer) (hemiptera:Aphididae) a species that causes severe economic losses due to its role as a vector of many plant viruses (Blackman and Eastop, 2000; Minks and Harrewijn, 1989). We studied the olfactory responses of *Sphaerophoria rueppellii* (Wiedemann) the main predatory syrphid in Mediterranean greenhouse crops and a promising aphid control agent in such agricultural systems (Amorós-Jiménez et al., 2012; Pineda and Marcos-Garcia, 2008) and specifically focussed on the possible avoidance of the odour of conspecifics, as well as *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), a coccinellid that is frequently commercialised for its use as a biocontrol agent in greenhouses. In olfactometer tests we studied the choices made by gravid *S. rueppellii* females and second-instar larvae when offered the volatile compounds emitted from unoccupied aphid-infested plants and plants carrying conspecific or heterospecific individuals or only their tracks. We also studied the acceptance of oviposition spots by gravid *S. rueppellii* females that could also see and contact conspecific and heterospecific competitors to corroborate the results from the olfactometer trials.

2. Material and Methods

2.1. Olfactometers

The attraction of female hoverflies to different odour sources was tested in a six-arm olfactometer described by Turlings et al. (2004), which allows up to six odours to be tested simultaneously for their relative attractiveness. The attraction of *S. rueppellii*'s larvae towards different odours was studied in a modified version of the 4-arm olfactometer described by D'Alessandro and Turlings (2006).

2.2. Plant material, aphids and predators

Plants: Sweet-pepper plants (*C. annuum* var. California Wonder) were grown from seed in a climate room (T = 25°C ± 1°C, RH = 60% ±10%, Photoperiod = 12L:12D) in plastic pots (5 x 5x 6 cm). These conditions were used throughout all of the rearing in this work. Aphids: A base colony of *M. persicae* was maintained on sweet pepper plants in mesh-netting insect-proof cages (30×30×30 cm; Megaview Science, Taiwan). Predators: (a) Syrphids: Adults of *S. rueppellii* originally came from a colony kept at the CIBIO research institute (Alicante, Spain). Adults were reared in similar cages as explained above, containing multi-flower bee pollen, sucrose, water and an aphid-infested pepper plant as oviposition stimulus. Syrphid larvae were maintained on *M. persicae* colonies on sweet pepper plants in similar cages. (b) Coccinellids: *A. bipunctata* larvae were obtained from Koppert Biological Systems (The Netherlands) and maintained until adults on sweet pepper plants infested with *M. persicae*, in similar cages as for the syrphids.

2.3. Odour sources

All the plants used in the experiments were 9 weeks old (6-7 extended leaves). When aphid-infested plants were required, sweet pepper plants were infested with 2-3 adults of *M. persicae* two weeks before the start of the experiment, in order to get a colony of approximately 400 individuals at the start of the trials. Aphid-infested plants containing immature stages and larval tracks from syrphids or coccinellids were also used as odour sources in some tests. To obtain plants with predator eggs, sweet-pepper plants were placed in a rearing cage containing mature adults of one of the two predators enough time to obtain 50 eggs per plant, and after that time plants were removed from the cage and kept without predators in separate glass vessels inside a climatic chamber. To obtain plants carrying larvae, 5 larvae of either *S. rueppellii* or *A. bipunctata* (of second and third instar, respectively) were carefully placed on the infested plant with a soft paintbrush. To prepare larval tracks, five larvae of either *S. rueppellii* or *A. bipunctata* were placed on a circular piece of parafilm (8cm diameter), placed in a petri dish. The Petri dish was sealed with parafilm and kept in a rearing chamber for 24h. After this time, the larvae and all the remains were carefully removed, and the parafilm disc was then attached to an infested plant. All odour sources were prepared on the evening before the start of the experiment, and after that plants were transplanted into a glass pot with a ground-glass male connector that was inserted into an odour source vessel (see Turlings et al., 2004).

2.4. Olfactometer bioassays

Bioassays were carried out with both larvae and female adults of *S. rueppellii*. Only those insects that made a choice were included in the analysis of the results (this represented 45% of the individuals tested).

Larvae: 4 – 5 day-old larvae (second-instar) were removed from their cage with a soft paint brush and carefully placed at the central choice chamber of the olfactometer. Because syrphid larvae are commonly nocturnal in their activity (Hopper et al., 2011), all the tests with this stage were done in dark conditions, as illumination induces escape, but no foraging behaviour (Amorós-Jiménez, pers, observ.). In the experiments that included plants as odour sources, the olfactometer plants were illuminated during 30 minutes previous to releasing the larvae in order to ensure volatile emission by plants (Gouinguene and Turlings, 2002).

Adults: Mated 6-7-day-old females were used and all test insects were naïve in that they had never encountered a prey or plant as an adult. They were removed from their cage with an aspirator and directly placed in a vial attached to a glass frit at the bottom of the central choice chamber of the olfactometer.

When attracted by an odour, the insect would crawl or fly into an arm through which such an odour entered the central chamber. In the arms their path was blocked by a stainless steel screen, and eventually, they walked up into an insect trapping bulb, where they could be counted and removed. All insects were given 30 min to make a choice, after which they all were removed and a new group was released. Each replicate consisted on three groups of either four or six individuals (see sections below). On a given day, all insects were tested with the same odour sources, which remained in the same position. The positions were kept this way because the odours can adsorb to the glass surface and arms could remain attractive even after removal of the odour sources (Turlings et al., 2004). For this reason, after each experiment all parts of the olfactometers (glass and Teflon) were cleaned thoroughly with distilled water and then with acetone and pentane at the end of each day. After the solvents had evaporated, the glass parts were placed overnight in an oven at 250 °C.

2.4.1. Intraspecific interference

To test the possibility that syrphids affect each other's choices by either attracting or repelling one another, the following preliminary experiment was conducted separately for females and larvae. Ten syrphids were placed in one of the arms for 1h. Plugs of cotton were used to prevent them from moving out of the arm. Through this procedure, odours emitted by the insects could adsorb onto the glass in the arm. After 1 h, the plugs and the insects were removed and the normal experimental airflows were passed through all arms. The other arms were left empty. On each test day, three groups of six larvae or three groups of four female adults were released in the choice chamber and their choices were recorded. This was repeated six times, each time with the 10 insects in a different arm.

2.4.2. Attraction to aphid-infested plants

The attractiveness of aphid-infested plants to syrphid larvae and adult females was first assessed in order to test if the olfactometer was suited for such assays and to determine if syrphids are able to distinguish an aphid-infested plant from a uninfested plant, which would be essential for the next experiments.

Larvae: An aphid infested sweet pepper plant was placed in one of the arms of a four-arm olfactometer, while a non-infested plant was positioned in a different arm. The two remaining arms were left empty.

Adults: Two infested and two non-infested sweet pepper plants were placed in different arms of the six-arm olfactometer, leaving the remaining two empty.

Each day, three groups of six larvae or three groups of four female adults were released in the choice chamber and their choices were recorded. This was repeated six times, with the odour sources being introduced through different arms each time.

Although observations in these two preliminary experiments showed that the great majority of the larvae did crawl into one of the four arms, they kept coming back to the central chamber and exploring new arms, showing no clear preference for a particular odour source. Figures 1A and 2A illustrate the last choice of larvae after they stopped searching. Based on the results of these experiments we decided to not test the response of syrphid larvae to volatiles from conspecific or heterospecific cues.

2.4.3. Effect of conspecific and heterospecific volatile cues

To assess the effect of conspecific volatile compounds on *S. rueppellii* female choices, two arms with only clean air were alternated with arms containing an aphid infested plant with eggs, larvae or larval tracks from the same hoverfly species. The remaining arm consisted of an infested plant without syrphids. Three times, six naïve female adults were released per replicate with a total of six replicates, and their choices were determined 30 min after release. For each replicate, the odour sources were placed in a different position.

To test the effect of heterospecific volatiles on host plant selection of *S. rueppellii* females, we followed a similar procedure as for the previous experiment, but in this case aphid-infested plants with eggs, larvae or larval tracks of *A. bipunctata* were used as odour sources.

2.5. Oviposition preferences

To study the effect of potential competitors on acceptance by *S. rueppellii* females of the oviposition sites, similar to the ones used in the olfactometer bioassays, the following multiple-choice experiment was carried out. An arena consisting on an insect-proof cage (30 x 30 x 60 cm) was placed inside a climatic chamber with the same conditions explained above. Four different oviposition sites were placed in different

corners of the cage and their relative position was randomised for every new replicate: An infested sweet-pepper plant was offered to the hoverflies as a positive control, while three infested plants with eggs, larvae or larval tracks of *S. rueppellii* were used to test the oviposition preference in the presence of conspecific predators. To evaluate the influence of heterospecific competitors, a similar procedure was followed.

A 6-7 day old mature syrphid female was placed in the centre of the cage for 24h. After this time plants were checked by visual inspection and the number of eggs laid on each plant was counted. Each fly was used only once and they had never experienced the test conditions before. Fifteen replicates were performed for each predator.

2.6. Statistical analysis

Olfactometer choices were analysed using a log linear model that takes into account overdispersion (including effects of insects influencing each other's choices) as described elsewhere (D'Alessandro and Turlings, 2006). Data from the oviposition behaviour experiment (proportion of eggs laid) were 2arcsin square-root-transformed to achieve homocedasticity prior to apply a Kolmogorov-Smirnov normality test. A one-way ANOVA test was then performed to study the ovipositional preference of syrphid females, followed by a Tukey post-hoc test for multiple pair-wise comparisons. All data were analysed with the statistical packages R (<http://stat.ethz.ch/CRAN/>) and SPSS V20.0. (IBM Co., NY, USA).

3. Results

3.1. Olfactometer bioassays

3.1.1. Intraspecific interference

Syrphid preferences did not seem to be positively or negatively influenced by the choices of their own kind. For both larvae and female adults, many of the individuals did not enter an arm (45% and 56%, respectively) and those that did distributed themselves evenly over all arms (*larvae*: $df = 1, 70$; deviance ratio = 0.91; $P = 0.32$; *adults*: $df = 1, 106$; deviance ratio = 1.25; $P = 0.21$), but there was a tendency for the adult females to avoid the arm with conspecifics (Fig. 1).

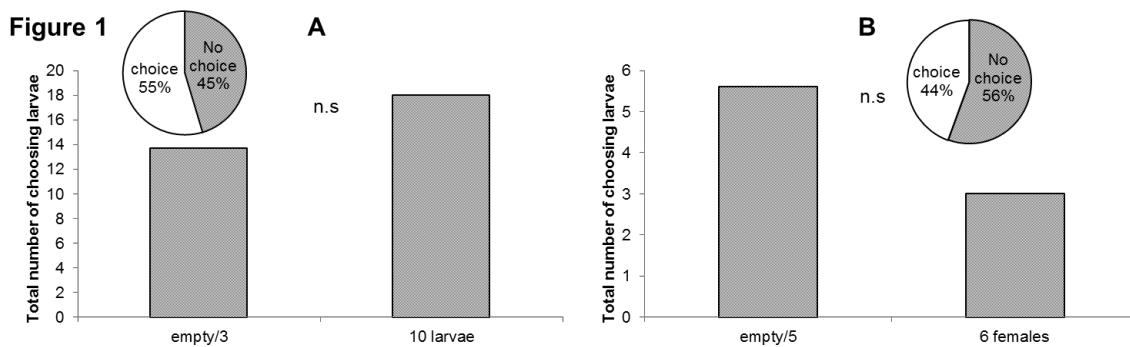


Figure 1. Responses of syrphids to the odour of conspecifics. The pie chart shows the percentage of insects that entered an arm. Choices for the control arms were summed and divided by three or five, depending on the experiment. (A) Choices made by second-instar larvae (B) Choices made by female adults.

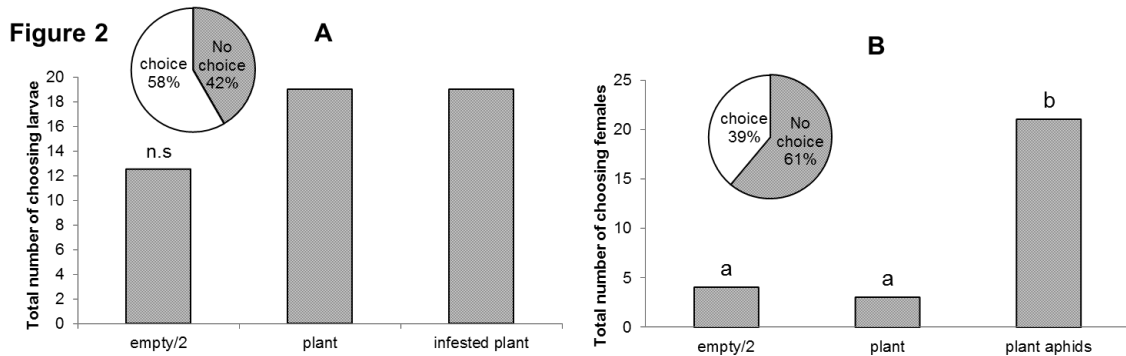


Figure 2. Attraction of syrphids to aphid-infested plants odours in a four-arm olfactometer. The pie chart shows the percentage of insects that entered an arm. Choices for the two control arms were summed and divided by two. Different letters indicate significant differences between the treatments ($P < 0.01$). (A) Choices made by second-instar larvae (B) Choices made by female adults.

3.1.2. Attraction to aphid-infested plants

This olfactometer test had a different outcome depending on which stage of *S. rueppellii* was used. While the larvae that entered an arm (58%) distributed uniformly over all four arms ($df = 2, 69$; deviance ratio = 2.70; $P = 0.39$) (Fig. 2A), the great majority of choosing females (39%) selected arms with infested plants over non-infested plants or empty arms ($df = 2, 105$; deviance ratio = 20.47; $P = p < 0,001$) (Fig 2B).

3.1.3. Conspecific and heterospecific volatile cues

In the first experiment, the response of naïve *S. rueppellii* females to infested plants with different conspecific cues as odour sources was tested. Only 37% of the flies made a choice, while 63% remained in the central chamber of the olfactometer. The great majority of the choosing females chose the arm with an infested plant without predator cues over the empty arm or the arm with an infested plant plus syrphid larvae ($df = 4, 103$; deviance ratio = 11.25; $P < 0.05$; Tukey test: $P < 0.01$). Although there

were not statistically different, females tended to choose an unoccupied infested plant over infested plants with syrphid eggs or larval tracks. There was no evidence of different behaviour among replicates ($df = 1, 102$; deviance ratio = 1.35; $P = 0.22$) (Fig. 3A).

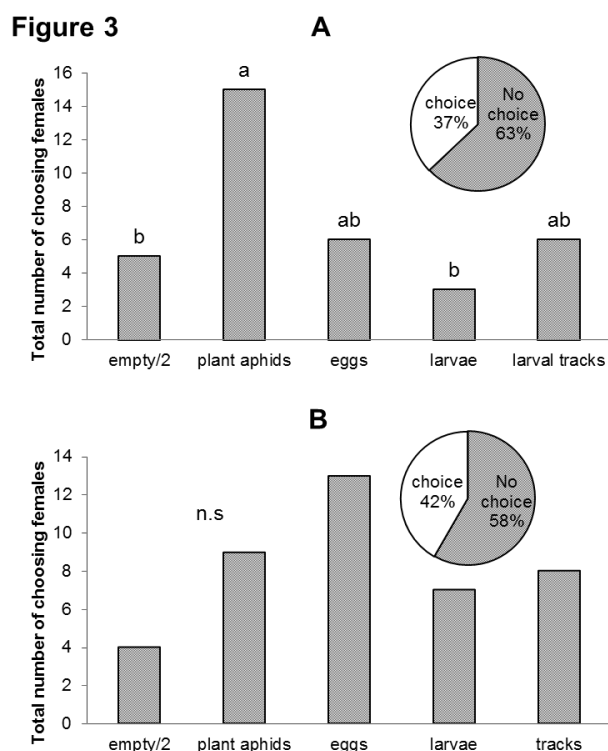


Fig 3. Olfactometer choices in the presences and absence of conspecific and heterospecific cues. The pie chart shows the percentage of syrphid females that entered an arm. Choices for the two control arms were summed and divided by two. Different letters indicate significant differences between the treatments ($P < 0.01$). (A) With odours of *S. rueppellii*. (B) with odours of *A. bipunctata*.

In the second experiment, we tested the response of naïve adult females to infested plants with odours from immature coccinellid stages. In this case, 42% of the released females made a choice, while 58% of them remained in the central choice chamber. There were no statistical differences ($df = 4, 103$; deviance ratio = 7.59; $P = 0.11$), although a higher number of flies chose an arm with an infested plant plus coccinellid eggs followed by an unoccupied infested plant, while a lower number of

females chose an arm with no odour or with an infested plant plus coccinellid larvae or larval tracks (Fig. 3B).

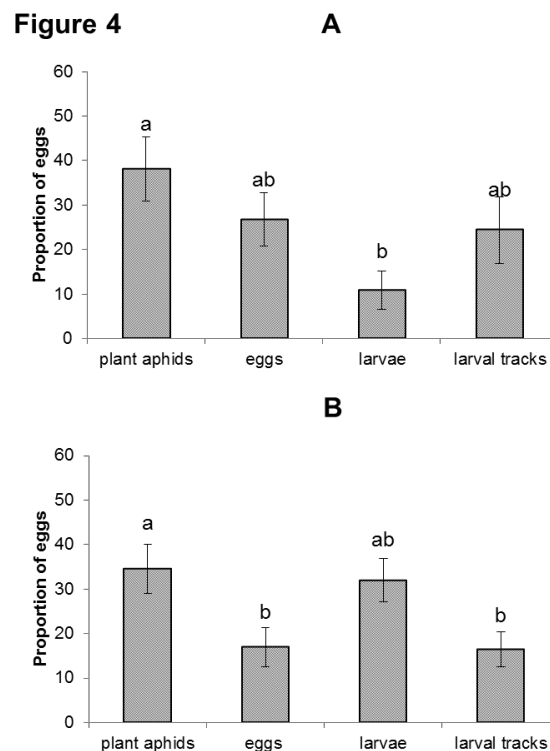


Figure 4. Proportion of eggs (Mean \pm SE) laid by *S. rueppellii* females in the presence and absence of conspecific (A) and heterospecific competitors (B). Different letters indicate significant differences among treatments (ANOVA and Tuckey test, $P < 0.05$).

3.2. Oviposition preferences

The proportion of eggs laid by syrphid females was influenced by the presence of conspecific immature stages ($df = 3, 47$; $F = 3.46$; $P = 0.024$). Females preferably laid their egg load on infested plants without syrphid immatures compared to those with syrphid larvae (Tuckey test, $P < 0.05$), while no significant differences compared to plants with syrphid eggs or larval tracks were found (Fig. 4A).

In contrast, female adults faced with coccinellid immatures laid fewer eggs on infested plants with coccinellid eggs and those with larval tracks, as compared to the

other two treatments ($df = 2, 59; F = 4.50; P < 0.07$; Tuckey test, $P < 0.05$). Unexpectedly, there were not statistical differences in the proportion of eggs laid on unoccupied infested plants and those laid on infested plants with coccinellid larvae (Fig. 4B).

4. Discussion

Olfactometer bioassays were conducted to explore whether volatile compounds are important for syrphid larvae and adults to detect and orient themselves towards food sources and oviposition sites, respectively. Similar experiments were then carried out to study if the odours of intra- and interspecific competitors affect the egg-laying preferences of *S. rueppellii* females. Females were found to effectively discriminate between aphid-infested plants and non-infested ones, relying on olfactory stimuli. Choice of oviposition sites in aphidophagous hoverflies is a complex process that involves visual, auditory, olfactory and gustatory cues (Almohamad et al., 2009). Yet, volatile compounds emitted from aphids and host plants can be sufficient to stimulate egg-laying in females even in absence of the other cues (Almohamad et al., 2008a; Verheggen et al., 2008). Indeed, most females in our olfactometer trials showed abdominal protraction and oviposition after entering an arm with an infested plant, despite the fact that they did not perceive anything other than olfactory stimuli. These observations highlight the key role that volatile semiochemicals play in decision-making behaviour of hoverflies. Green leaf volatiles (GLVs) and the aphid-alarm pheromone [(E)- β -farnesene] have been recognized as important volatile stimuli for the location and acceptance of oviposition stimuli for syrphids and other aphid predators (Dicke, 1994; Francis et al., 2004; Verheggen et al., 2008).

In contrast to these positive results, plant and aphid volatiles did not attract *S. rueppellii*'s larvae, which distributed equally over the olfactometer arms, including both

infested and non-infested plants, as well as the ones carrying clean air. Literature on the role of semiochemicals in the foraging behaviour of syrphid larvae is scarce and results more or less support the notion that larvae do not use volatiles to locate aphids, or that they have limited importance for first instar larvae at short distances (Bargen et al., 1998; Francis et al., 2005). Instead, non-volatile (gustatory) compounds from, for instance, honeydew triggers specific larval locomotory behaviours (slowing, turning and ‘casting’) and seem to have a greater role in their prey-locating success (Bargen et al., 1998; Chandler, 1969). In our experiments, only second instar larvae were used, as first instars are hard to manipulate without causing extensive mortality and they require very high humidity conditions to remain active (Amorós-Jiménez et al., 2012). Whether first instar larvae of *S. rueppellii* use infochemicals to forage for aphids and in what extent they are important is a question that this work cannot answer. Females of the hoverfly *Episyrphus balteatus* are known to regularly lay their eggs on uninfested plants (Sadeghi and Gilbert, 2000), forcing the hatching larvae to forage for themselves. Indeed, larvae of this species are able to move long distances and survive long periods without encountering aphids (Rojo et al., 1996). In contrast to this strategy, *S. rueppellii*'s larval stages have a more limited movement capacity due to their lower tolerance to starvation periods, and females mostly lay their eggs close or even inside aphid colonies (Amorós-Jiménez, pers. observ.). We therefore hypothesized that *S. rueppellii* larvae rely greatly on the selection of suitable oviposition sites by their mothers, ensuring they have enough food to fully develop. This requires good detection of suitable sites by the adult females, but there is no need for sophisticated prey-location traits in the larvae as aphids are mainly found by chance contact.

The presence of conspecific volatiles ‘contaminating’ the odour blend from aphid-infested plants were apparently detected by *S. rueppellii*'s females, and rendered such odour sources less attractive, since females significantly preferred the non-contaminated infested plants. We also found a clear trend of lower numbers of *S.*

rueppellii females choosing plants with eggs or larval tracks than without them (Fig. 3A), but these differences were not supported statistically. Interestingly, the same results were obtained when females were allowed to oviposit on contaminated and non-contaminated aphid-infested plants (Fig 4A). The fact that the deterrent effect of eggs and larval tracks was less intense than with larvae present on the plant might be related to lower concentration of recently deposited oviposition-detering substances. Indeed, attractive stimuli associated with plants and aphids may overpower the repellent effects of volatiles from eggs and larval tracks of conspecific competitors (Almohamad et al., 2010b). Although it has already been established that detection of intraspecific competitors involves chemical cues derived from eggs, larvae or larval tracks (e.g Almohamad et al., 2010a; Almohamad et al., 2010b; Scholz and Poehling, 2000) our study reveals the specific importance of volatiles in these interactions, and shows that syrphids respond to them even in the absence of other stimuli.

Syrphid female choices were not affected by volatile emissions from immature stages of the coccinellid *A. bipunctata*. Although there was a tendency for a greater number of females preferring arms with a plant over an empty vessel (Fig. 3B), infested plants without coccinellids did not attract more females as we were expecting. Remarkably, the oviposition experiments produced the anticipated result: females reduced their oviposition rate in response to the presence of coccinellid immatures, although the differences between unoccupied plants and plants with larvae were not statistically supported (Fig. 4B). That syrphid oviposition was not significantly deterred by the presence of coccinellid larvae may be related to the high aphid-alarm pheromone production they induce. We know that *A. bipunctata* induces a stronger antipredator behaviour in *M. persicae* than *S. rueppellii* larvae, including an enhanced secretion of exudate from their cornicles, which contains the alarm pheromone (E)- β -farnesene (Belliere et al., 2011). This pheromone may enhance the attraction of natural enemies (Francis et al., 2004; Francis et al. 2005). When larvae are no longer present

(larval tracks treatment) and the production of the alarm pheromone has stopped, this added attractiveness ends, possibly explaining the differences observed in syrphid's oviposition preferences.

Previous works on the avoidance by hoverfly females of aphid colonies with heterospecific predators support these results (Almohamad et al., 2010a; Almohamad et al., 2009; Hindayana et al., 2001; Laubertie et al., 2006; Putra et al., 2009). Specifically, for Coccinellidae it has been demonstrated that chemical cues released by larval stages play a role in this avoidance behaviour (Almohamad et al., 2010a). Yet, our results suggest that the sole perception of related volatiles is not deterrent enough for syrphid females. Possible explanations are that adult syrphids do not rely significantly on semiochemicals to detect heterospecific competitors, or that volatiles elicit a response only when they are combined with other stimuli. For example, Almohamad et al. (2010a) found that *E. balteatus* females laid fewer eggs on leaf discs contaminated with *Harmonia axyridis* larval tracks and that this involved a mixture of volatile and gustatory stimuli as well as visual perception of the plant tissue. In another study (Putra et al., 2009), *E. balteatus* female response to coccinellid larvae varied depending on the instar they encountered, again suggesting that syrphid's response may rely on visual cues.

It is interesting that *S. rueppellii* females responded strongly to conspecific volatiles, whereas they did not respond to volatiles from *A. bipunctata*. This is similar to what has been found for some coccinellidae, where females were not deterred from ovipositing in the presence of heterospecific cues (Almohamad et al., 2010a; Yasuda et al., 2000). As they use the same resource, aphidophagous predators may converge on the same plants (Agarwala and Yasuda, 2001), but differential environmental requirements may keep them in a particular habitat or seasonal period, possibly reducing the chances of encountering each other and limiting competition (Honek, 1983; Honek and Kocourek, 1988). For instance, Pineda and Marcos-Garcia (2008)

found a temporal succession of the syrphid species composition in Mediterranean greenhouses, which was attributed to the influence of temperature and humidity conditions on the species' phenology. *Sphaerophoria rueppellii* was the most abundant species under high temperatures and dry conditions, when few other aphid predators are present (Amorós-Jiménez et al., 2012; Pineda and Marcos-García, 2008). It is therefore likely that when *S. rueppellii* females are foraging for oviposition sites, they encounter plants with individuals from the same species much more frequently than those with other syrphid species. Due to the low occurrence of *A. bipunctata* in Mediterranean agricultural areas (supplementary Table 1), we expect the same to apply to this heterospecific competitor, and this limited co-occurrence (Putra et al., 2009) could explain the weak response of *S. rueppellii* to its volatiles.

The present study reveals an important role for volatile semiochemicals in the foraging behaviour of syrphid flies and their selection of oviposition sites. It also shows a differential importance of volatiles in the avoidance of plants that carry conspecific and heterospecific predators. Information on the identity of such volatiles is scarce. Hemptinne and Dixon (2000) found that coccinellid larvae and larval tracks release a mixture of alkanes and Almohamad et al. (2010b) identified a blend of acids, alcohols and aldehydes in the emissions from *E. balteatus* larvae and their tracks. Identifying the specific volatile-compounds that are involved in the recognition of conspecifics by *S. rueppellii*, as well as further studies on the possible perception of other heterospecific competitors – including other syrphid species – will be important to obtain a full understanding of the ability of this predator to detect and avoid intraguild competitors.

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Capítulo 4

Feeding preferences of the aphidophagous hoverfly *Sphaerophoria rueppellii* affect the performance of their offspring

Las preferencias tróficas del sírfido afidófago *Sphaerophoria rueppellii* afectan al éxito de su descendencia

Amorós-Jiménez, R., Pineda, A., Fereres, A., Marcos-García, M. A., 2013. Feeding preferences of the aphidophagous hoverfly *Sphaerophoria rueppellii* affect the performance of their offspring. Submitted to Biocontrol.

Abstract

1. Provision of additional floral resources in the crop is a strategy that has proven successful for several natural enemies including predatory syrphids. However, the selection of flower species for the attraction of natural enemies is mainly based on visiting preferences, paying little attention to the relation between these preferences and the effects on performance and fitness.

2. In this study, we assess the influence of feeding on flowers of two insectary plants (sweet alyssum and coriander) and a crop species (sweet pepper) on fitness-related parameters of the parental and first generation of the syrphid *Sphaerophoria rueppellii*.

3. We found that adult nutrition affected the performance of the offspring, and that the adult floral preference was linked to an optimum performance of the next generation. Sweet alyssum was the flower most frequently visited by syrphid adults, and adult body size and egg-to adult survival were enhanced when parents fed on this flower species.

4. The second preferred species was coriander, and feeding on this flower remarkably enhanced the oviposition of *S. rueppellii* females compared to the other flower species.

5. Our study showed that each flower species had different effects on various performance parameters of *S. rueppellii*, highlighting the importance of understanding these effects for the success of habitat management strategies.

1. Introduction

Habitat management through the addition of flowering plants in and around crops is a strategy of conservation biological control that enhances the performance of natural enemies (Landis et al., 2000). The importance of floral resources for a large number of natural enemies such as many parasitoid species as well as predators like Coccinellidae, Chrysopidae and Syrphidae have been widely reported (Jervis et al., 1993; Al-Doghairi and Cranshaw, 1999). Predatory hoverflies (Diptera: Syrphidae) have a high potential as biocontrol agents against aphids (Tenhumberg and Poehling, 1995; Brewer and Elliott, 2004; Freier et al., 2007; Haenke et al., 2009). Several works have shown that this family of diptera respond positively to the addition of flowering plants to the crop, which can increase their residence time and their efficiency as biocontrol agents, resulting in a lower aphid density and crop damage (Hickman and Wratten, 1996; Pineda and Marcos-García, 2008a; Haenke et al., 2009).

Floral resources may have a significant effect on egg production of females and other fitness related parameters of syrphids (Scholz and Poehling, 2000), since adults rely on proteins from pollen and saccharide from nectar to mature their ovaries and sustain egg production and to obtain energy, respectively (Chambers, 1988; Gilbert, 1993). Therefore, understanding the effect that different species of floral resources have on syrphids is crucial when applying a habitat management strategy of aphid control (Macleod, 1992; Cowgill et al., 1993). When evaluating floral resources for habitat management strategies in outdoor crops, traits that enhance attractiveness are desired. However, when introducing flowering plants in greenhouses, enhancing the performance and fitness of natural enemies can be as desirable as improving their attraction. Surprisingly, very few works have paid attention to the effects of flowering plants on syrphid fitness (Laubertie et al., 2012), and even less studies have been performed on the link between fitness and syrphid foraging behaviour (Laubertie et al., 2012). Due to this fact, criteria for selecting flower resources to enhance syrphid

populations have been based mainly on the floral preferences of syrphid adults. In addition, these preferences differ between syrphid species (Haslett, 1989), highlighting that for the success of habitat management implementation is key to understand the effects of floral resources for the targeted natural enemy.

The present study aims to show the relation between the attractiveness of flower resources and their effect on hoverfly fitness-related parameters of both the parental and the first generation. To address this objective, we compared two of the most commonly used species of flowers that have been successfully applied in habitat management strategies to enhance the populations of natural enemies: sweet alyssum (*Lobularia maritima* L. Desv.) and coriander (*Coriandrum sativum* L.). Furthermore, we included sweet pepper (*Capsicum annuum* L.) as an additional non-host resource, to evaluate the importance that the crop itself may have on conservation biological control. The syrphid species we chose to conduct these work is *Sphaerophoria rueppellii* Wiedemann (Diptera: Syrphidae) the most abundant predatory hoverfly in Mediterranean greenhouse crops (Pineda and Marcos-García, 2008b). This species is well adapted to the extreme climatic conditions occurring in these agricultural areas (Amoros-Jimenez et al., 2012), and it has previously shown a positive response to habitat management strategies including the provision of additional floral resources (Pineda and Marcos-García, 2008c) and banker plants (Pineda and Marcos-García, 2008). Encouraging results in aphid density reduction and in the improvement of the production quality in field conditions (unpublished results) make this syrphid a promising biocontrol agent of aphids in greenhouses.

2. Material and Methods

2.1. Plant material, aphids and syrphids

Sweet pepper (var. California Wonder), sweet alyssum and coriander plants were grown from seed in plastic pots (5 x 5 x 6 cm) in a glasshouse under controlled conditions and natural light (25°C ± 2°C, RH = 60% ±10%, Photoperiod = 12L:12D). Flowering plants were sown in a weekly basis to assure there were fresh flowers throughout the experiment. Due to the fact that time to flowering varies among species, time of sowing was different for each flower species in order to synchronise their flowering. A stock colony of *M. persicae* was maintained on sweet pepper plants in mesh netting cages (30 x 30 x 60 cm) in a climate room under controlled conditions (T = 25°C ± 1°C, RH = 60% ±10%, Photoperiod = 14L:10D). *Sphaerophoria rueppellii* cohorts were reared in plastic cages (30 x 30 x 60 cm), with a fine mesh on its upper part with the same environmental conditions. The rearing cages contained bee pollen from various flowers, sucrose, water, and a sweet pepper plant infested with *M. persicae* to stimulate oviposition. Same-aged syrphid eggs were periodically removed from the adult cultures and the emerged larvae were reared until pupation on sweet pepper plants infested with *M. persicae*.

2.2. Syrphid floral preference

Relative attractiveness of the three flowering species was evaluated in a multiple-choice experiment. The arena consisted on an insect-proof cage (40 x 40 x 50 cm) located inside a rearing chamber with the same conditions explained above. One flowering plant of each species was placed in one of the corners of the cage. Relative position of each plant was randomised for every replicate to account for possible differences in light intensity caused by the structure of the chamber. A 2-4 day old adult syrphid (male or female) was placed in the centre of the cage, inside of a glass tube

covered with black adhesive tape to help orientating the syrphid towards the tube opening. Each fly was used only once and they had never experienced the test conditions before. Using the behavioural observation program Etholog (Ottoni, 2000) the time devoted to feed on each flower species and the frequency of syrphids visiting each flower were recorded. Observations lasted for 10 minutes. Adults that did not exhibit any recognition behaviour (i.e. the syrphid performs a suspended flight near the plant or lands on it) during this time were discarded. Twenty replicates were performed for each sex. Tests were always conducted from 9 to 11 am.

2.3. Fitness-related parameters in the parental generation

To assess the effect of feeding on different flower resources on *S. rueppellii*'s performance, one newly-emerged female and two males were placed in a plastic cage (30 x 30 x 60 cm), with a fine mesh on its upper part with the same conditions as for insect cultures. Each cage contained a non-flowering sweet pepper plant infested with approximately 300 individuals of *M. persicae* as oviposition stimulus, water and one of the flowering plants to be tested. Flowers and infested plants were replaced every few days to ensure syrphids had continuous access to fresh pollen and nectar as well as aphids. Each cage was daily checked by visual inspection for syrphid survival and oviposition. The eggs laid on the infested-pepper plants were counted to evaluate syrphid fecundity (i.e. number of eggs every 24h). To quantify fertility (i.e. fraction of eggs hatching), leaves containing eggs were cut off from the plant, and placed in a Petri dish (90 x 15 mm) with a wet disk of paper inside, and sealed with Parafilm® to achieve a high relative humidity, necessary for egg stage to complete (Amorós-Jiménez et al., 2012). Counts and collection of eggs stopped after fifteen days, as the optimal oviposition period for this species is within this range, and after this period of time egg-laying remarkably decreases (Amorós-Jiménez, pers. observ.). Survival was

measured until all the syrphids had died. Ten replicates were performed for each flower species.

Adult life span, time to sexual maturation, total fecundity and fertility were measured as components of syrphid's fitness.

2.4. Fitness-related parameters in the 1st generation

To evaluate a possible effect of adults feeding habits on the performance of their offspring, eggs with the same age were obtained by placing an aphid-infested sweet pepper plant in a rearing cage containing approximately 100 mature adults for two hours. This cage also contained water and 2-4 plants of one of the tested flower species, which were periodically replaced for fresh ones. Eggs were reared in optimal conditions (see plant material, aphids and syrphids), and they were daily checked for egg-hatching. Recently-emerged larvae were individualised in a Petri dish (90 x 15 mm) with a piece of a sweet pepper leaf (1 cm²), a wet disk of paper to provide moisture, and sealed with Parafilm[®]. Ad libitum food (*M. persicae*) was supplied throughout the larval cycle. The sweet pepper disc was replaced daily, to ensure that the aphids had access to fresh food throughout the experiment. Larval mortality and developmental time of larvae were assessed by observing each Petri dish every 24h, from a total of 50 larvae per treatment.

Once larvae pupated, the pupae were kept under the same conditions until adult emergence. From the emerged adults wing length was determined, which is a good indicator of fitness in many diptera families (Stubbs and Falk, 1983). Specifically, the shortest length between the junction of the costal vein (C) with the humeral crossvein (h) and the junction of R4+5 with the medial vein (M) was measured (Dziocik, 2005). Due to differences between males and females, measurements were analysed

separately for each sex and always using the same wing (right or left), to avoid any effect due to asymmetry.

2.5. Statistical analysis

Data on wing length were normally distributed and therefore were analysed with a two-way ANOVA, followed by Tukey post-hoc test for multiple pair-wise comparisons. A Kaplan-Meier survival analysis was used to evaluate the effect of 'sex' and 'flower species' on adult survival in the parental generation (Hosmer and Lemeshow, 1999), and log-rank test was used for pairwise comparisons. All other data were not normally distributed and were subsequently analysed with Generalized Linear Models (GLM), and LSD post-hoc test for multiple pairwise comparisons. For the parameter of visit frequencies, we adjusted the model to a Binomial distribution with logit link function with 'sex' and 'flower species' as the main factors, while 'cage' was included as a nested term to 'flower species'. Time devoted to feed on each flower as well as data on fitness-related parameters of parental generation (total fecundity and fertility, time to sexual maturation and adult life span) and developmental time of larvae and pupae in the first generation, were all analysed assuming a Poisson error structure of data and a log relationship between the response variable and the explanatory variables. A binomial error structure of data and logit link function was used for adult emergence rate. All data were analysed with the statistical packages SPSS V20.0 and Gen Stat 13.

3. Results

3.1. Syrphid floral preference

The average number of visits by syrphid adults was significantly different for the three flower species tested ($df = 2, 107$; deviance ratio = 9; $P < 0.001$), with sweet alyssum being the species more frequently visited followed by coriander and sweet pepper in the last position (LSD test: $P < 0.05$) (Fig.1A). No other significant sources of variation could be found. The same pattern of preference was showed when the time feeding on each flowering plant was studied ($df = 2, 107$; deviance ratio = 12.36; $P < 0.001$) (Fig.1B). The factor sex did not have a significant effect on the number of visits nor on the feeding time.

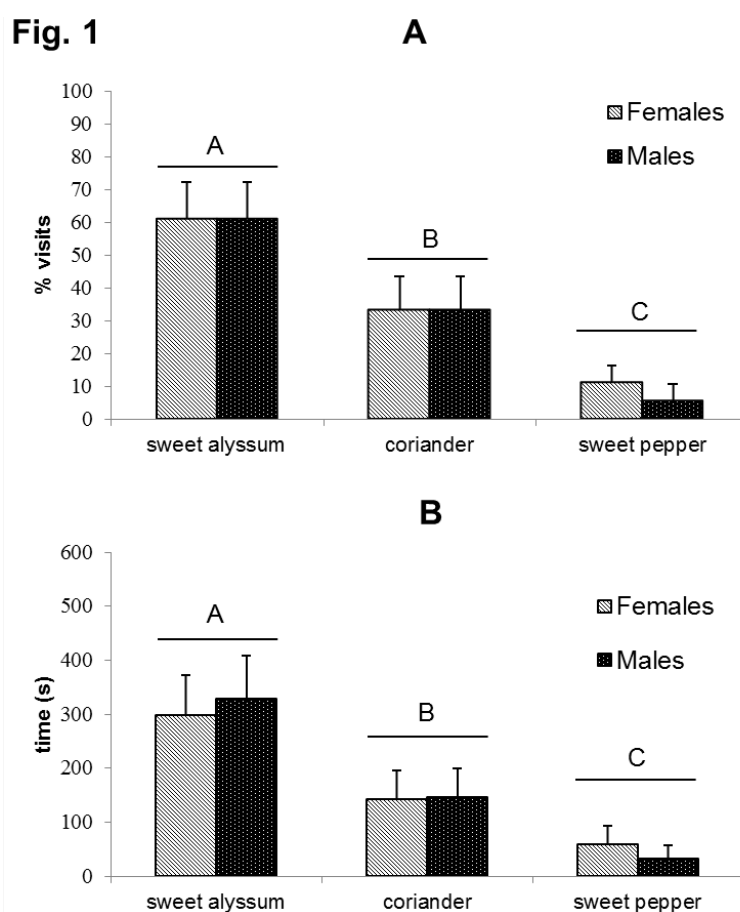


Figure 1: Syrphid floral preference. Mean \pm SE of the percentage of flower visits (A) and feeding time (B) of male and female syrphid adults on each flower species. Different letters above bars indicate significant differences (GLM, LSD).

3.3. Fitness-related parameters in the parental generation

Feeding on different flower resources had a strong impact on the number of eggs laid by *S. rueppellii*'s females. The analysis showed that total fecundity over 15 days was significantly higher for syrphids that were fed with fresh coriander flowers (df = 2, 28; deviance ratio = 15.63; $P < 0.001$) (Fig.2A) whilst there were no statistical differences between sweet alyssum and sweet pepper (LSD test: $P > 0.05$). The GLM did not show a significant effect of flower species on total fertility (df = 2, 28; deviance ratio = 1.61; $P = 0.219$), and time to sexual maturation (df = 2, 29; deviance ratio = 0.49; $P = 0.618$) (Table 1). None of the sources of variation from the analysis of adult life span were significant (data not shown). After observing that sex did not influence adult life span, the effect of flower species was analysed using the Kaplan-Meier survival analysis, showing that they do not affect syrphid survival (log-rank; $\chi^2 = 0.66$, df = 2, $P = 0.717$) (Fig.2B).

3.4. Fitness-related parameters in the 1st generation

Flower species had a differential effect on the developmental time of *S. rueppellii* larvae (df = 2, 109; deviance ratio = 69.7; $P < 0.001$), which showed a faster development when the parental adult fed on sweet pepper plants, followed by sweet alyssum, and finally coriander (LSD test: $P < 0.05$) (Table 1). In contrast, pupal developmental time was not affected by the flower species (df = 2, 109; deviance ratio = 2.04; $P = 0.136$). Interestingly, the flowering plant species that the parents ingested had a significant effect on the survival of the next generation. The percentage of syrphids that succeeded on becoming adults was significantly higher on the sweet alyssum treatment compared to the other flowers (df = 2, 149; deviance ratio = 4.13; $P = 0.018$, LSD test: $P < 0.05$) (Fig.3A). In the same way, flower species markedly influenced the body size of these emerging adults (df = 2, 101; $F = 3.85$; $P = 0.025$),

since feeding on sweet alyssum increased wing length of both sexes compared to coriander and sweet pepper, although statistical differences could only be found between sweet alyssum and coriander treatments (Fig.3B). Females showed a greater wing length than males regardless of the flower species ($df = 1, 101$; $F = 117.56$; $P < 0.001$), a pattern that has already been shown in previous studies with the same species (Amoros-Jimenez et al., 2012).

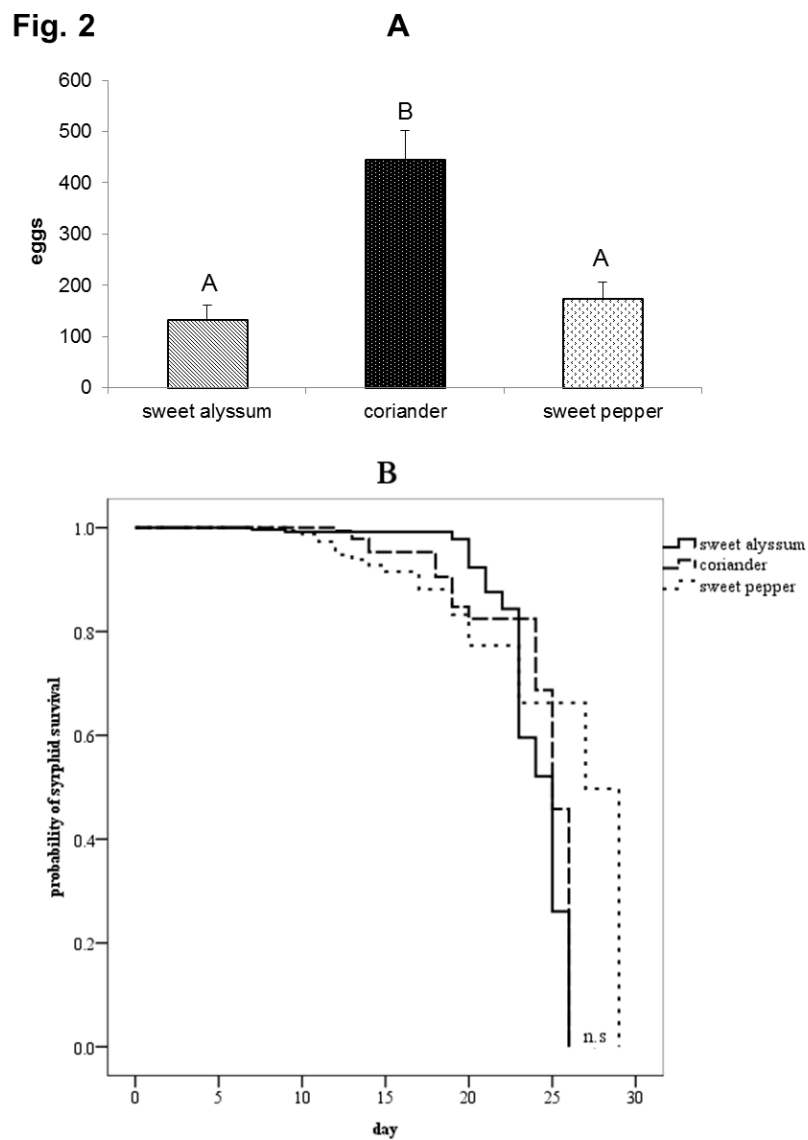


Figure 2: Fitness-related parameters of the parental generation. Mean \pm SE of total fecundity within 15 days (GLM, LSD) (A) and probability of survival of syrphid adults feeding on each flowering plant (Kaplan-Meier, Log-rank test) (B). Different letters above bars indicate significant differences.

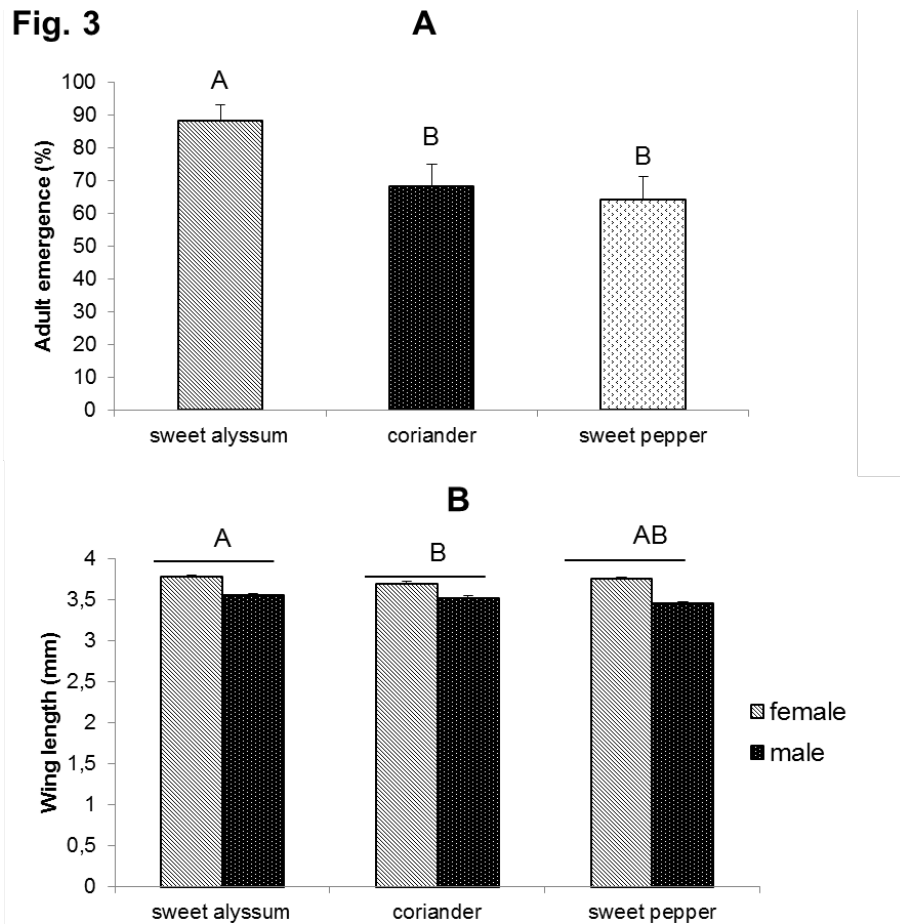


Figure 3: Fitness-related parameters of the 1st generation. Mean \pm SE of adult emergence rate (%) (GLM, LSD) (A) and wing length (mm) of male and female adults (Two-way ANOVA, Tuckey test) (B) fed with each flowering plant. Different letter above bars indicate significant differences.

		sweet alyssum	coriander	sweet pepper
parental generation	total fertility (%)	71.64 \pm 6.91a	58.53 \pm 4.35a	54.89 \pm 6.66a
	sexual maturation time (days)	3.8 \pm 0.31a	3.5 \pm 0.29a	3.4 \pm 0.29a
1st generation	development time larva (days)	8.2 \pm 0.09a	9.47 \pm 0.11b	7.69 \pm 0.11c
	development time pupa (days)	5.11 \pm 0.07a	5.18 \pm 0.07a	5.31 \pm 0.08a

Table 1: Effect of each flowering plant on various fitness components of the parental and 1st generation (Mean \pm SE). Treatments labeled with the same letter are not significantly different (GLM, LSD).

4. Discussion

The present work reports for the first time the effects that several flowering plants have on some fitness-related parameters of the aphid predator *S. rueppellii*. Coriander increased *S. rueppellii*'s fecundity in a greater extent, in terms of daily fecundity and total fecundity after fifteen days. Ambrosino et al. (2006) found that this was the flower species that most effectively augmented the proportion of *Episyrphus balteatus* (De Geer) females that were successful in laying eggs, although oviposition rate was not significantly boosted. On the contrary, neither coriander nor the other flower species seemed to influence other fitness components of *S. rueppellii* such the egg-hatching rate, sexual maturation or adult longevity. Previous studies have shown an effect on survival of syrphid adults depending on the floral resources that were supplied (Laubertie et al., 2012).

Nevertheless, the most interesting result is the evidence that parental adult diet has an effect on offspring performance in syrphids. Parents fed with sweet pepper flowers produced larvae with a reduced time of development, shortening thus generation time. Sweet alyssum, in turn, had a positive effect in the offspring's percentage of emerged adults, increasing this parameter in a minimum of 20% compared to the other flowers. Furthermore, these adults had a larger body size, a parameter that is correlated with a large number of species' attributes that influence their fitness, including longevity, reproductive rate or resource use (Harvey and Pagel, 1991; Blackburn and Gaston, 1994). Several works have already demonstrated that dietary conditions experienced by parents can be transmitted to the next generation, a transgenerational influence known as parental effect (Wolf and Wade, 2009). For instance, Valtonen et al. (2012) showed that the larval diet of *Drosophila melanogaster* influenced their offspring development time, adult body size and even adult resistance to pathogens. The underlying mechanism for this 'transmission' is thought to act through maternal mRNA's that are transferred into the unfertilised eggs, or through

post-zygotic influences via maternal traits such as nutritional provision (Wolf and Wade, 2009). In our case, it is possible that nutrients or other substances dependent of the syrphid adult's diet are transferred to eggs or embryos by the female, influencing certain traits of the offspring that constitute a fitness benefit. These advantages are not apparent until the later stages of development, in the form of an enhanced egg-to adult survival and adult body size.

Our study also provides the first report of a link between *S. rueppellii*'s floral preference and the enhanced performance of their offspring. Sweet alyssum was the flower most frequently visited by *S. rueppellii* adults of both sexes; coriander was intermediate while sweet pepper flowers were visited in a lesser extent. It is interesting that once the syrphid visited a flower species, practically in all the individuals tested, it did not leave that particular plant throughout the foraging-behaviour records. As a consequence, the time that syrphid adults devoted to feed on sweet alyssum flowers was also the greatest, followed by coriander. According to the results on floral preference and fitness-related parameters of the offspring, *S. rueppellii* females are able to select those resources that will result on an enhanced fitness of the next generation. Both sweet alyssum and coriander have been previously pointed out as two of the most appropriate species for the attraction of syrphids (Colley and Luna, 2000; Ambrosino et al., 2006; Hogg et al., 2011), since both have flowers with short corolla that facilitates the availability of nectar and pollen, and white flowers which stimulate feeding in hoverflies (Colley and Luna, 2000; Vattala et al., 2006). Sweet pepper flowers, on the other hand, provide big pollen and nectar volumes, and floral rewards are also within easy access to pollinators (Rabinowitch et al., 1993). However, previous works have also reported a low attractiveness to honeybees and other insects, presumably due to the poor sugar concentrations found in flower nectar (McGregor, 1976; Baker and Baker, 1983).

Regardless, the provision of floral resources for natural enemies by the crop plants should be also taken in consideration, a topic that has already been addressed in previous works (Venzon et al., 2006). Despite the fact that fitness-related parameters were higher when syrphids fed on non-harvestable plants, it is clear that pollen and nectar from sweet pepper flowers is suitable for the development and reproductive success of *S. rueppellii*. Pineda and Marcos-García, (2008c) showed that sweet pepper pollen was always present in the gut of predator syrphids that naturally entered greenhouses even if flowering plants were present, and in some cases its presence in syrphid's diet was more important than the introduced flowers. This indicates that syrphids that come from the outside or are released in the greenhouse regularly consume pepper pollen, which in fact complements their nutrition and enhances fitness parameters (e.g. shortening the development time of larval stage) that the other flowers do not.

As we have previously stressed, both sweet alyssum and coriander are firmly accepted as suitable flowering plants for attracting hoverflies and other natural enemies. Focussing on their effects on *S. rueppellii*, although each flower species enhanced different components of syrphid's performance, sweet alyssum seems to be the species that most positively affects it. This species increases survival of the next generation and its fitness-related parameters (e.g. body size) in the greatest extent and thus, it should be the flowering plant to preferably use in habitat management strategies in agricultural areas where this syrphid is particularly important, such as Mediterranean greenhouses (Pineda and Marcos-García, 2008b). In addition, this flower fulfils important requirements determining the potential usefulness of an insectary plant, such as a long blooming period that ensures a continuous supply of pollen for the natural enemies, a low attractiveness towards pest herbivore species (Hogg et al., 2011) and a low potential to act as weed (Pineda, pers. observ.). Moreover, sweet alyssum seems to specifically attract hoverflies over honeybees and

other Apoidea, which due to their territoriality may prevent hoverflies from visiting flowers (Ambrosino et al., 2006) and so, using an insectary plant that predominantly attracts the target natural enemy may be an important factor for the successful provision of floral foods. Nevertheless, despite these desirable features a more realistic approach than using a single species is to combine several flower species with different phenologies or nutritional attributes (Colley and Luna, 2000).

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Capítulo 5

Effect of UV-absorbing nets on the performance of the aphid predator *Sphaerophoria rueppellii*

Efecto de las mallas absorbentes de UV en la acción del depredador de pulgón *Sphaerophoria rueppellii*

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Abstract

Photoselective nets have proven to be effective for aphid pest control as they limit their dispersal ability. However, little is known on the impact of such nets on natural enemies of aphids other than parasitoids. In this work we study for the first time the effect of UV-absorbing nets on the syrphid fly *Sphaerophoria rueppellii* Wiedemann (Diptera: Syrphidae), a potential aphid biocontrol agent in Mediterranean horticultural crops. First, we released mature syrphid adults and we evaluated density and dispersal of the resulting syrphid immatures in a turnip crop grown under either UV-blocking (Bionet) or standard net. Second, we assessed under controlled conditions the impact of UV radiation on fitness-related parameters and on flight behaviour of *S. rueppellii* adults. Results showed that the type of net had no influence on the performance of adults. However, immature density was higher, and dispersion lower, under Bionet. The effect UV-absorbing nets are known to have on the dispersion pattern of aphids may have indirectly caused these differences. We conclude that the use of photoselective nets and the release of syrphid predators such *S. rueppellii* are compatible strategies to be used in IPM aphid-control programs.

1. Introduction

Aphids (Hemiptera: Aphididae) are one of the most destructive and abundant pests of horticultural crops worldwide (Ramakers, 2004). The damage aphids cause through feeding and, more importantly, the large amount of honeydew they produce and their efficiency as vectors of plant viruses, make them one of the most damaging pests in protected crops (Blümel, 2004; Byers, 2008; Rabasse and Steenis, 2002).

In recent years, development of pesticide resistance by aphids has stimulated the implementation of Integrated Pest Management (IPM) strategies for aphid pest control, in both indoor and outdoor crops (Chyzik et al., 2003; Sanchez and Lacasa, 2006). One of such IPM strategies is the use of UV-absorbing covers that block part of the UV radiation transmitted inside greenhouses. UV-absorbing covers have proven to be effective at reducing the incidence of pests and diseases in commercial greenhouses (Ben-Yakir et al., 2008; Díaz and Fereres, 2007). Alteration of the UV part of the solar spectrum perceived by insects affects behavioural traits such as spatial orientation, navigation, feeding, and mating behaviour (Coombe, 1982; Scherer and Kolb, 1987). Recent studies showed that UV-deficient environments reduce winged aphids flight and reproduction ability, thereby reducing aphid propagation and dispersal rate within greenhouses (Chyzik et al., 2003; Legarrea et al., 2012b; Legarrea et al., 2012c). The success of IPM strategies involving the use of photoselective covers for aphid control requires not only the understanding of the effects of these covers on the insect pest and their host plants, but also on their natural enemies (van Lenteren and Noldus, 1990). Surprisingly, only few works have studied the effects of UV-blocking barriers on natural enemies of aphids, and all of them focused on parasitoids (Chiel et al., 2006; Chyzik et al., 2003). At present, the effects of these materials on aphid predators such as ladybeetles, lacewings or syrphid flies remain unknown (Díaz and Fereres, 2007).

This work aims at evaluating the effect of photoselective covers (i.e. UV-blocking nets) on the spatiotemporal dynamics of an aphid predator, the syrphid fly *Sphaerophoria rueppellii* Wiedemann (Diptera: Syrphidae). This species is the most abundant predatory syrphid in Mediterranean greenhouse crops, and, currently, it is one of the most promising biocontrol agents for aphid control in greenhouses (Amorós-Jiménez et al., 2012; Pineda and Marcos-García, 2008a). The experimental system involved turnip [*Brassica napus* L. (Cruciferae)] and sweet pepper [*Capsicum annuum* L. (Solanaceae)] plants infested with the aphid *Myzus persicae* Sulzer, a species that causes severe economic losses in numerous crops worldwide (Blackman and Eastop, 2000; Minks and Harrewijn, 1989). Field experiments and bioassays under controlled conditions were carried out to explore the effects of UV- blocking covers on fitness-related parameters, dispersal and foraging behaviour of the syrphid.

2. Material and Methods

2.1. Plant material, aphids and syrphids

Sweet-pepper [*C. annuum* L. (Solanaceae) var. California Wonder], turnip (*B. napus* var. Just Right) and sweet alyssum [*Lobularia maritima* L. (Brassicaceae)] plants were grown in a climate room (T = 22°C ± 2°C, RH = 80% ±10%, Photoperiod = 16L:8D) in plastic pots (5 x 5x 6 cm). Sweet-pepper plants were used to maintain a stock colony of *M. persicae* in mesh netting cages (50 x 35 x 35 cm) in a climate room at the same conditions described above. *Sphaerophoria rueppellii* adults were also reared in mesh netting cages (60 x 60 x 80 cm) containing bee pollen from various flowers, sucrose, water, and a sweet-pepper plant infested with *M. persicae* to stimulate oviposition. Same-aged syrphid eggs were periodically removed from the adult cultures and the emerged larvae were reared until pupation on sweet-pepper plants infested with *M. persicae*.

2.2. Field experiments

Experimental design

Experiments were conducted in “La Poveda”-CSIC, an experimental farm located in Arganda del Rey, Madrid, Spain (40°18'N, 3°26'W). Two “tunnel type” nethouses (6.5 m wide, 8 m long and 2.6 m height) with the same orientation (N-S) and separated five meters from each other were used to compare two types of nets: a standard 50 mesh net (Criado y López, El Ejido, Spain) with no special UV-absorbing properties, and Bionet (Meteor Agricultural Nets, Ltd., Petach-Tickva, Israel) which filters about 40% radiation in the UV spectrum. These two nets have similar physical properties (mesh size, open area, mechanical strength). The two nethouses contained four compartments (6.5 m wide, 2 m long and 2.6 m height), each one isolated from the adjacent with walls made of standard 50 mesh net. Compartments constitute the experimental units of the experiment. Because alternating the two types of nets in the compartments within each nethouse could cause undesirable shading from adjacent covers with photoselective properties of the other treatment, all four compartments of each nethouse were covered with the same type of net. Nested models were applied to account for potential effects derived from such experimental design (see ‘Statistical analysis’).

To corroborate that UV-blocking differed between the two types of nets, the UV radiation transmitted inside each compartment was measured before every insect count using a portable quantum meter radiometer sensitive to ultraviolet radiation (320–400 nm) (Apogee, Logan, USA). Temperature and relative humidity inside each compartment were registered using data loggers (Tinytag Ultra 2s, Gemini Data Loggers, Chichester, UK). These measures were taken because climate conditions inside greenhouses are a key factor influencing the performance of natural enemies, since it determines establishment and reproduction of adults, as well as development

and survival of immature stages (Shipp and Van Houten, 1997; Zhang and Shipp, 1998).

In each compartment, 66 turnip plants at the 2-leaf stage were transplanted in early September 2010, distributed in eleven rows. Plants were spaced 0.3 m along water drip rows, and 0.5 m between rows. On the same day, five flowering sweet alyssum plants were transplanted in the corners and the centre of each compartment, to serve as food source for syrphid adults. This plant species is very attractive for hoverflies, and several works have demonstrated its suitability as insectary plant (Laubertie et al., 2012; Pineda and Marcos-Garcia, 2008b). One week after transplanting, a Petri dish containing 80-100 *M. persicae* winged adults was placed in the centre of each compartment to promote a natural infestation into the crop. An initial density of 5-10 aphids per plant in each compartment was achieved a week later. At this moment, three mature 7 day-old females and two males of *S. rueppellii* were released in each compartment. These syrphids came from a mass-reared colony originally started at the CIBIO research institute (Alicante, Spain), and maintained as described above.

Aphid and syrphid samplings

Monitoring of aphid and syrphid population was conducted twice a week from 24th September to 8th November 2010. At the beginning of sampling, turnip plants were at the growth stage 16, according to the extended BBCH scale (Meier, 1997). The number of aphids and syrphid immature stages (eggs and larvae) were counted by thorough inspection of 7 turnip plants randomly selected. Aphid abundance on each plant was grouped into five categories depending on population density, following the same scale as described in Legarrea et al., (2012b): 0: 0 aphids; 1: 1-4 aphids; 2: 5-19 aphids; 3: 20-49 aphids; 4: 50-149 aphids; 5: ≥ 150 aphids. Aphid stages (nymphs, apter or alate adults) were not counted separately because egg production in female

hoverflies depends on prey availability, not on their stage structure (Hemptinne et al., 1993). Indeed, *S. rueppellii*'s larvae successfully develop into adults when they are let to feed on all aphid stages (Amorós-Jiménez & Marcos-García, unpublished results).

2.3. Effects of UV on fitness-related parameters and foraging behaviour

The experiments were carried out in insect-proof cages allocated inside a glasshouse under controlled conditions ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $\text{RH} = 60\% \pm 10\%$, Photoperiod = 12:12), at the ICA-CSIC (Madrid). Cages were covered with either photoselective or standard nets. Because light naturally entering the glasshouse strongly depended on the local weather, UV values registered inside the two types of cages often did not differ. To better mimic radiation during sunny days, three special lamps (ULTRA-VITALUX, Osram, Spain) producing light similar in its composition to the mixture of natural sunlight were placed above the cages as additional light sources. As a result, the percentage of UV radiation transmitted inside standard-netting cages was in average 38% higher than that measured under the photoselective nets, in all the experiments (Supplementary Table). Cages were placed on top of a table (5 m long x 1.81 m wide) oriented N-S. Relative position of cages, and orientation on the table, was randomised on a daily basis to account for possible differences in light intensity caused by the structure of the glasshouse, or by shadows from other structures outside the glasshouse. UV radiation was measured using the same device described above.

Fitness-related parameters

To assess the effect of photoselective covers on *S. rueppellii*'s fecundity and fertility, one newly-emerged female and two males were placed in a mesh-netting cage (40 x 40 x 40 cm) covered by either Bionet or standard net. Preliminary studies revealed that the size of the cages is suitable to perform flight and choice experiments (Amorós-Jiménez et al. unpublished results). Each cage contained food, water and a sweet-pepper plant infested with approximately 300 individuals of *M. persicae* at the

start of the trials. Syrphids were left seven days in these conditions to ensure maturation and mating. After this period, each plant was observed by visual inspection to confirm that oviposition had taken place, and females that had not laid eggs were discarded. Each mature female was transferred to a cage covered with standard mesh that contained a new aphid-infested plant. After 48 h, the eggs laid on this plant were counted to evaluate syrphid fecundity (i.e. number of eggs in 48h). To quantify fertility (i.e. fraction of eggs hatching), leaves containing eggs were cut off from the plant, and placed in a Petri dish (90 x 15 mm) with a wet disk of paper inside, and sealed with Parafilm® to achieve the high relative humidity conditions necessary for egg development to complete (Amorós-Jiménez et al., 2012). Ten replicates were performed for each treatment.

Foraging behaviour

To test the effect of UV-blocking materials on the recognition of floral resources by adults, we used mesh-netting cages (100 cm long, 60 cm wide and 60 cm height) covered with either the standard 50 or the Optinet 50 mesh (Polysack Plastic Industries, Ltd., Nir Yitzhak, Israel), the latest with similar UV-absorbing properties than the Bionet 50 mesh. A flowering sweet alyssum plant was placed in one of the extremes of the cage. A 2-4 day old syrphid female was placed in the opposite side of the cage, inside of a glass tube placed on a flying platform 30 cm above the ground. The tube was covered with black adhesive tape to help syrphids to orientate towards the tube opening. Each female was used only once and they were naïve, in the sense that they never experienced the test conditions before. The time devoted to recognise the floral resource (i.e. time until the syrphid performs either a suspended flight near the plant or flies in circles around it) was recorded using the behavioural observation program Etholog (Ottoni, 2000). Observations lasted a maximum of 15 minutes. Females that did not exhibit this behaviour during the interval were also included in the statistical analyses (see below). Ten replicates were performed for each cover.

2.4. Statistical analysis

UV radiation, temperature and relative humidity under each type of net were analysed separately with Generalized Linear Models (GLM), which included two main factors: 'nethouse' and 'compartment' nested to 'nethouse'. We assumed a Normal error structure of data and a logit relationship between the environmental data and the lineal combination of the explanatory variables.

The abundance of aphids (scale value) and syrphid immature stages on each type of nethouse were analysed using Generalized Linear Mixed Models (GLMM), which allow for non-linearity, non-constant variance, and clustered structure of data, including time as random variable, the main factor 'compartment' nested to 'nethouse', and assuming a Poisson error structure of data and a logit relationship between the response variable and the explanatory variables (Agresti and Natarajan, 2001; McCulloch and Searle, 2004). Additionally, data on presence-absence of syrphid immatures on turnip plants were analysed with GLMM adjusted to a Binomial distribution with logit link function, as proxy for the dispersion of immature stages within each type of net.

Fecundity and fertility data followed a normal distribution and were analysed with t-tests. Fertility data were log-transformed to achieve homoscedasticity. To detect whether nets interfered differentially with the foraging behaviour of syrphids, the time needed by each individual to recognize flowers was compared between type of nets using Kaplan-Meier survival analysis (Hosmer and Lemeshow, 1999). This analysis permits the inclusion of replicates in which the event (flower recognition) does not occur (i.e. censored data - (Hosmer and Lemeshow, 1999). A log-rank test was used for pairwise comparisons (Hosmer and Lemeshow, 1999). All data were analysed with the statistical package SPSS V20.0. (IBM Co., NY, USA).

3. Results

3.1. Field experiments

Light properties and environmental conditions

UV radiation was significantly higher under the standard net than the Bionet (22.82 ± 0.78 and $10.61 \pm 0.35 \mu\text{mol}/\text{m}^2 \text{ s}$, respectively: $\chi^2 = 220.97$, $df = 1$, $p < 0.001$). There were no differences in UV radiation among compartments within each nethouse ($\chi^2 = 8.92$, $df = 6$, $p = 0.18$).

Mean daily temperature was significantly lower under the photoselective than under the standard cover (15.77 ± 0.31 and 17.36 ± 0.31 , respectively: $\chi^2 = 4.94$, $df = 1$, $p = 0.03$), a result that agrees with previous works (Dixon, 1988; Legarrea et al., 2012b), whilst there were not statistical differences among compartments within each nethouse ($\chi^2 = 3.01$, $df = 4$, $p = 0.56$). Despite the average temperature under the two nets was below the theoretical range for optimal development and performance of *S. rueppellii* (Amorós-Jiménez et al., 2012), both adults and immatures seemed to be well adapted to the nethouse conditions. Indeed, this species is able to locate and exploit microhabitats within plants that provide shelter and optimal abiotic conditions (Amorós-Jiménez et al., 2012).

The relative humidity was not different between the two types of net (68.29 ± 0.86 and 69.73 ± 1.05 , respectively: $\chi^2 = 1.12$, $df = 1$, $p = 0.29$), neither among compartments within nethouses ($\chi^2 = 0.49$, $df = 3$, $p = 0.92$). Average relative humidity of all compartments was always above the critical value for *S. rueppellii* immature development (i.e. 60%) (Amorós-Jiménez et al., 2012).

	Standard	Photoselective
Field experiment	48.93 ± 1.30a	20.39 ± 0.88b
Fitness-related parameters	99.46 ± 7.56a	61,63 ± 5.01b
Foraging behaviour	87.04 ± 6.01a	49.29 ± 4.43b

Supplementary table: Percentage of UV radiation transmitted under each type of net (mean ± SE). Different letters between Standard and Photoselective refer to significant differences (student t-test, P < 0.05).

	Source of variation	F	df	P
Aphid density	Nethouse (1)	36.572	1, 768	< 0.001
	Compartment(nethouse) (2)	3.854	6, 768	0.001
	Time (3)	578.189	1, 768	< 0.001
	(1) * (3)	19.483	1, 768	< 0.001
	(2) * (3)	1.315	6, 768	0.248
Syrphid egg density	Nethouse (1)	4.167	1, 623	0.042
	Compartment(nethouse) (2)	1.414	4, 623	0.228
	Time (3)	2.697	1, 623	0.101
	(1) * (3)	0.001	1, 623	0.976
	(2) * (3)	0.530	4, 623	0.714
Syrphid egg presence-absence	Nethouse (1)	0.000	1, 77	0.999
	Compartment(nethouse) (2)	0.551	5, 77	0.737
	Time (3)	0.000	1, 77	1.000
	(1) * (3)	0.000	1, 77	1.000
	(2) * (3)	0.168	5, 77	0.974
Syrphid larvae density	Nethouse (1)	5.368	1, 525	0.021
	Compartment(nethouse) (2)	2.224	5, 525	0.051
	Time (3)	50.918	1, 525	<0.001
	(1) * (3)	1.539	1, 525	0.215
	(2) * (3)	0.916	5, 525	0.470
Syrphid larvae presence-absence	Nethouse (1)	4.808	1, 63	0.032
	Compartment(nethouse) (2)	0.721	5, 63	0.610
	Time (3)	82.345	1, 63	<0.001
	(1) * (3)	1.396	1, 63	0.242
	(2) * (3)	1.229	5, 63	0.306

Table 1: Resume table of GLMM model

Density of aphids

The average density of aphids was significantly higher under the photoselective than under the standard net throughout the experiment (4.18 ± 0.05 and 3.78 ± 0.05 , respectively) (Table 1). However, this effect was probably caused by aphid densities being higher under the Bionet than under the standard net from the beginning (Fig. 1a). The analysis also detected differences in aphid density among compartments within nethouses (Table 1).

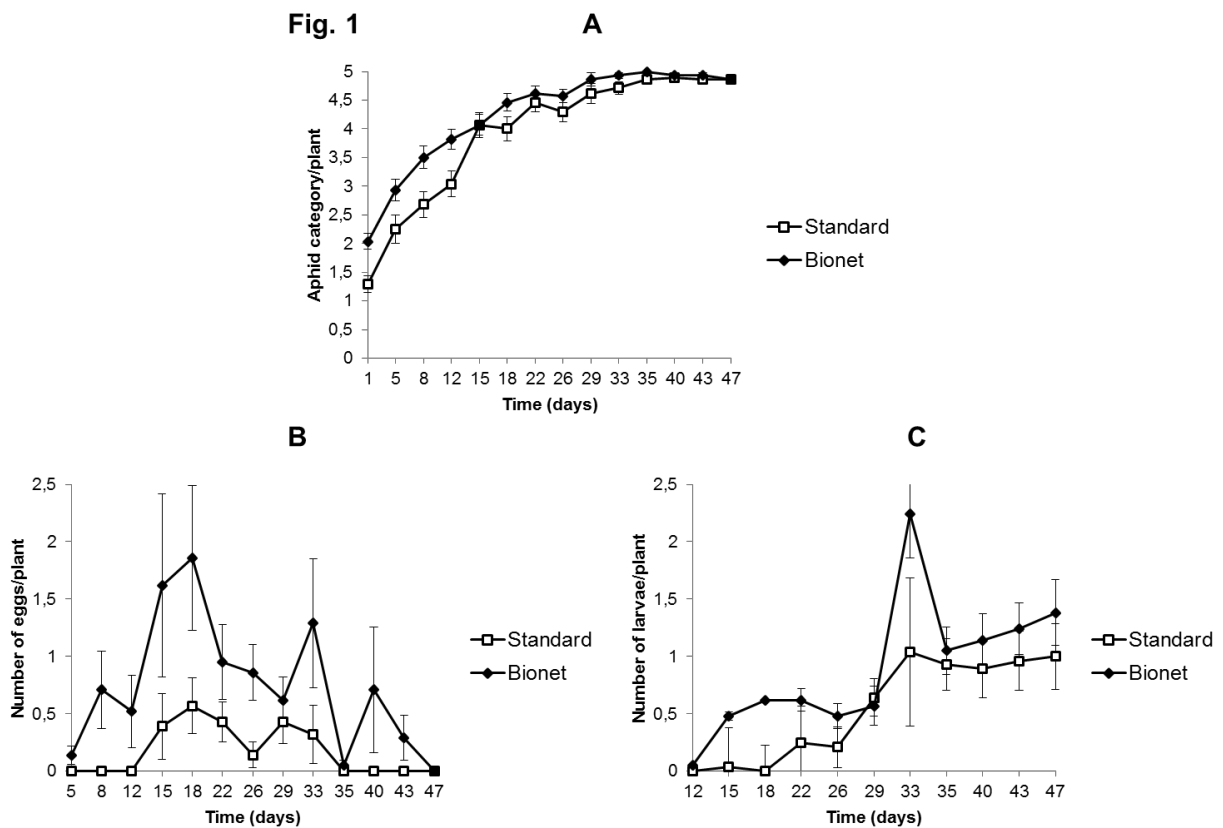


Figure 1: Mean \pm SE of the temporal evolution of aphid (scale value) (a) syrphid eggs (b) and larvae (c) counts in the nethouses covered with Bionet and Standard net.

Density of syrphid eggs

The average number of eggs per plant was remarkably higher under the photoselective than under the standard cover (0.61 ± 0.09 and 0.18 ± 0.04 ,

respectively) (Table 1), whereas no other significant sources of variation were detected. Fig. 1b shows that syrphid egg-laying started a week earlier, and it lasted longer, under the Bionet than under the standard cover. None of the sources of variation from the analysis of the spatial distribution (presence/absence) of syrphid eggs were significant; consequently no conclusions could be made on dispersal of females based on egg distribution.

Density of syrphid larvae

The abundance of *S. rueppellii*'s larvae was also markedly higher under the photoselective than the standard net (0.71 ± 0.08 and 0.31 ± 0.05 , respectively) (Table 1). Larvae were observed earlier, and at higher densities, under the Bionet than on the standard net (Fig. 1c). Interestingly, the analysis of the spatial distribution of syrphid larvae revealed that under the standard net a higher number of plants had presence of larvae, relative to the UV-blocking net (Table 1), suggesting that dispersal of larval stages was higher under standard netting.

3.3. Effects of UV on fitness-related parameters and foraging behaviour

Fitness-related parameters

The number of eggs laid by mature females after 48 h was not significantly different between the two treatments (75.55 ± 13.56 standard and 60.5 ± 14.38 Bionet: $t_{19} = 0.76$, $P = 0.46$). Similarly, no significant differences in fertility were found (58.09 ± 6.58 standard and 44.5 ± 4.17 Bionet: $t_{19} = 1.61$, $P = 0.12$).

Foraging behaviour

The proportion of syrphids that displayed flower-recognition behaviour did not differ between nets. Despite plant recognition was faster under the standard than the

photoselective net (Fig. 2), the probability of plant recognition by syrphids along time did not differ between treatments ($\chi^2 = 1.54$, $df = 1$, $P = 0.21$) (Fig. 2).

Fig. 2

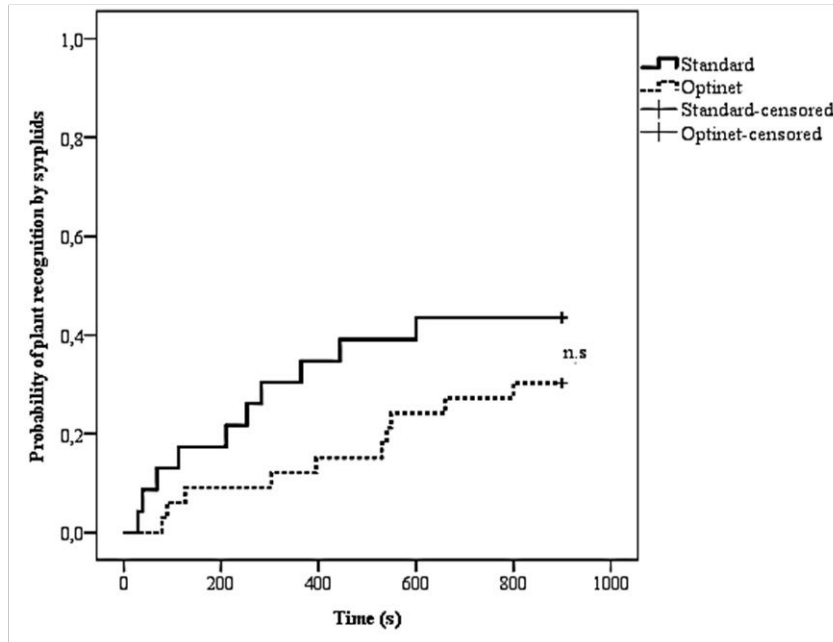


Figure 2: Cumulative proportion of syrphid females recognising the flowering plant (sweet alyssum) (Kaplan-Meier) under Optinet ($n = 33$, drawn line) and Standard net ($n = 23$, dotted line)

4. Discussion

Field and lab experiments were carried out to determine whether UV radiation influenced the performance of the aphidophagous syrphid *S. rueppellii*. In our experiments, the density of both egg and larval stages of *S. rueppellii* was higher under the Bionet mesh, suggesting that photoselective covers may enhance fecundity or fertility of the released females. Yet, life-history experiments indicated that these traits were not influenced by the lack of UV radiation. Density-dependent oviposition may provide an alternative and more likely explanation. In syrphids, oviposition site choice is known to depend on multiple factors, such as host plant characteristics, aphid species, presence of other predators, and prey abundance (Almohamad et al., 2009). Several

studies have shown that syrphids adjust the number of eggs laid to the size of the aphid colonies, to maximize the survival probability of their offspring (Almohamad et al., 2006; Belliure and Michaud, 2001; Chandler, 1968). In fact, aphid depletion before larvae complete their development might result in death by starvation and/or cannibalism (Almohamad et al., 2009). Higher number of immature stages found under the Bionet mesh may have been caused by female syrphids adjusting their oviposition rate to the denser aphid colonies found under these nets. Indeed, aphid density was consistently higher under the photoselective than the standard net, although probably due to an initial higher density of aphids under Bionet. This higher density would also imply higher presence of volatiles and honeydew produced by the aphid colonies, which are known to stimulate oviposition in syrphids (Almohamad et al., 2008; Budenberg and Powell, 1992). Furthermore, syrphid eggs were not observed until aphid density reached the same category of infestation under both nets (20-49 aphids per plant), what may indicate that at lower aphid densities the stimuli provided by the prey do not surpass the acceptance threshold of *S. rueppellii* females (Courtney et al., 1989).

Our results also suggested that syrphid larvae dispersed more under the standard net. Syrphid larvae are able to move between plants in search for new aphid colonies (Banks, 1968), and dispersion is often triggered when aphid availability within a plant is insufficient to support larval development to maturity (Kan, 1988a; Kan, 1988b). In addition, it is known that UV-blocking nets have a negative effect on dispersal and propagation of aphids, through a lower production of alate forms and a reduced motor activity (Chyzik et al., 2003; Legarrea et al., 2012c), what leads to higher aphid aggregation patterns in crops under UV selective nets (Legarrea et al., 2012a). We hypothesized that because the standard netting does not interfere with aphids' dispersal, syrphid larvae probably found smaller aphid colonies under the standard nets, what in turn triggered their moving between plants in search for new food

patches. Indeed, *S. rueppellii* larvae are able to survive under low aphid availability conditions, by exploiting small and developing aphid colonies in different plants. (Amorós-Jiménez et al., 2012).

Syrphid females require proteins and amino acids from pollen for their ovaries to mature and to sustain egg production, and nectar to gain energy to search for oviposition sites (Chambers, 1988). Therefore, factors hampering syrphids to find flowers could easily result on lower syrphid density in the crop. In this work we also evaluated whether UV-blocking nets reduced the ability of syrphid adults to find flowering plants they use as food sources. Our results suggested that this was not the case, as the probability of adult syrphids to find flowers did not differ under the two types of nets. However, our results are not conclusive onto whether UV-blocking nets affect syrphid visual perception of flowers, as syrphids are still capable of using other senses to locate food sources. Indeed, while optical stimuli are important for syrphid choice-making behaviour (Chandler, 1966), volatile compounds also play a key role in the attraction of syrphids towards flowering plants (Colley and Luna, 2000). At the greenhouse's scale, syrphids are in close contact with plants and their herbivores, and such olfactory cues might be more important than visual cues for hoverfly detection of, and orientation towards, their food sources, as well as their oviposition sites.

Knowing the degree of compatibility between biological control agents and the use of UV- absorbing covers is crucial for successful pest management in protected crops (Legarrea et al., 2012c). Our results suggest that UV- deficient environments induced under photoselective screens do not have a negative effect on population dynamics or performance of the predator *S. rueppellii*. Instead, UV- radiation indirectly influences the abundance, and shapes the distribution of this predator within the crop, as it affects the dispersal of their prey. As it has been shown in other studies, while natural enemies that mainly rely on their vision may have difficulties on their host/prey searching behaviour under UV-deficient environments (Chiel et al., 2006; Legarrea et al., 2012c),

predatory syrphids are able to efficiently find aphid patches attracted by the blend of volatiles produced by plants and by aphid colonies (Verheggen et al., 2008). Our results suggest that they may be able to do so also under low UV radiation environments. Based on our results, we can conclude that the use of UV-absorbing nets and syrphid releases are compatible strategies to be incorporated into IPM programs against aphid pests.

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Capítulo 6

Discusión general y resultados

La presente tesis ha estado dedicada al estudio del sírfido afidófago *Sphaerophoria rueppellii*, una especie abundante en el área mediterránea y cuyo potencial para convertirse en un eficaz agente de control de pulgones en esta área ha sido ya apuntada en varios trabajos previos, aunque hasta el momento no se había llevado a cabo una investigación dirigida a profundizar en el conocimiento de este depredador. Uno de los motivos por los que actualmente se piensa que la investigación en sírfidos afidófagos y su aplicación comercial como agentes de control es menos importante que la de otros insectos, es el escaso conocimiento que se tiene sobre su biología, debido en parte a que sus larvas son difícilmente localizables en las plantas y a que su actividad depredadora es predominantemente nocturna. La falta de un conocimiento profundo sobre cualquier agente de control, lleva con frecuencia a un manejo inadecuado del mismo y por lo tanto, a su ineficacia en el campo. En otras ocasiones, conduce al uso de especies poco idóneas para determinadas áreas geográficas, cultivos, tipos de manejo o para determinados periodos fenológicos. Uno de estos desafortunados ejemplos ha sido el uso que hasta el momento se ha dado a los sírfidos en los agroecosistemas mediterráneos.

Nuestro trabajo parte de la necesidad de obtener información rigurosa para el correcto manejo y aplicación como agente de control biológico de *S. rueppellii*, así como el resultado de su combinación con diferentes estrategias de control en vistas a su posible uso en el control biológico aumentativo contra pulgones. A continuación se presentan y discuten los resultados obtenidos, según los objetivos planteados al inicio.

- La primera parte de nuestro estudio estuvo encaminada a conocer diversos aspectos de la biología del sírfido y las interacciones que éste establece con sus áfidos presa y con otros agentes de control con los que coincide espacio temporalmente en su área de distribución:

Hemos demostrado que *S. rueppellii* está adaptada a un amplio rango de temperaturas, incluyendo **temperaturas elevadas** similares a las que se alcanzan en los invernaderos mediterráneos. Además de desarrollarse con normalidad y completar su ciclo biológico en dichas condiciones, lo hace más rápidamente que a temperaturas moderadas, lo que es beneficioso para su acción como agente de control biológico mediante liberaciones inoculativas en invernaderos mediterráneos, al acortarse el tiempo entre generaciones. Por el contrario, esta especie no se desarrolla adecuadamente cuando es expuesta a **baja humedad ambiental**, situación típica de los periodos de sequía en el sureste español. Estos resultados, junto con algunas observaciones de campo nos llevan a pensar que el hecho de que *S. rueppellii* sea la especie de sírfido afidófago que mejor tolera estas condiciones se debe a adaptaciones predominantemente comportamentales y no fisiológicas.

Sphaerophoria rueppellii presenta una **voracidad similar**, aunque ligeramente inferior, a la especie de sírfido comercialmente disponible por el momento (*Episyrphus balteatus*), siendo esta ligera diferencia atribuible al menor tamaño de la primera. Sin embargo, esta menor voracidad puede ser compensada desde el punto de vista de su eficacia como agente de control biológico por una característica muy interesante que hemos identificado, como ha sido el ser capaz de **reducir su tasa de consumo** de pulgones en condiciones de escasez de presas. Como consecuencia, las larvas son capaces de completar normalmente su desarrollo aunque la disponibilidad de alimento sea limitada. Esta cualidad les otorga el potencial para explotar los focos incipientes de pulgón en los cultivos y controlarlos desde su comienzo, lo que constituye una de las claves para un eficaz control biológico de cualquier plaga.

Concluimos que *S. rueppellii* es un depredador cuyo especial interés como agente de control de pulgones recae en el hecho de su adaptación para desarrollar su actividad adecuadamente a temperaturas elevadas, iguales o superiores a 30°C. A pesar de no presentar una mayor voracidad que su especie homóloga, es la única especie de sírfido que puede liberarse con expectativas de éxito, y que está presente de forma natural en el interior de los invernaderos y en las zonas adyacentes a los cultivos, en los meses más calurosos. Todo esto unido a su capacidad para sobrevivir con una baja abundancia de presas (situación habitual al inicio de la colonización del cultivo por pulgones), hace que este depredador sea un buen candidato para el control de pulgones durante la época más calurosa en los cultivos mediterráneos, momento en el cual atacan severamente a los cultivos.

El estudio del comportamiento de oviposición de las hembras de los agentes de control es otro aspecto crucial de la investigación en control biológico de plagas. Los estadios inmaduros de muchos de estos agentes son relativamente sésiles, y su supervivencia depende de la adecuada selección de los lugares de oviposición por parte de las hembras, maximizando la disponibilidad de alimento pero evitando a la vez la **competencia intragremial**. Es el caso de los sírfidos, que cuentan con una fase larvaria relativamente poco móvil comparada con otros grupos del mismo gremio como coccinélidos o neurópteros, y que además de ser depredadas también corren riesgo de ser canibalizadas por sus congéneres. Se trata pues de una búsqueda que va mucho más allá de la simple localización de presas, en la cual los estímulos químicos presentan un papel importante.

En esta tesis hemos evaluado la importancia que los **compuestos volátiles** tienen en el comportamiento de oviposición de las hembras de *S. rueppellii* frente a la presencia de competidores intragremiales potenciales,

así como la relevancia de estos semioquímicos en la búsqueda de presas durante su fase larvaria, aspecto del que apenas se tiene datos en esta familia de dípteros. A pesar de que ya era bien conocido que los sírfidos evitan ovipositar en colonias de pulgones donde otros sírfidos están presentes, hemos demostrado por primera vez el **papel clave de los estímulos olfativos** induciendo esta respuesta comportamental. Esto es una característica muy deseable para su acción como agente de control, puesto que en el cultivo, las hembras evitarán ovipositar cerca de aquellas colonias de pulgón donde detecten, ya sea mediante la vista o valiéndose de semioquímicos, la presencia de otros sírfidos, explotando nuevos focos de pulgón que todavía no hayan comenzado a ser controlados. Puesto que hemos visto que los compuestos volátiles no presentan un papel importante en la detección de las colonias de pulgón durante la fase larvaria, que además es ciega, el éxito como agente de control de esta especie recae en esta búsqueda llevada a cabo por las hembras adultas.

Por el contrario, hemos demostrado que se produce una respuesta de evasión muy débil ante volátiles del coccinélido *Adalia bipunctata*, y son necesarios estímulos adicionales (visuales, gustativos y táctiles) para que las hembras del sírfido muestren el comportamiento esperado descrito en la bibliografía. Nuestros estudios previos de campo indican que *A. bipunctata* es un coccinélido poco abundante de manera natural en nuestras zonas agrícolas, lo que hace pensar que su coincidencia espacio temporal con el sírfido está muy restringida, dado que este último está precisamente asociado a estas áreas. Creemos que esta baja coexistencia puede ser la responsable de que en esta particular interacción no se dé un reconocimiento químico entre ambas especies. Estos resultados ponen en evidencia no solamente la importancia de utilizar especies nativas, sino de usar agentes de control que

se encuentren de forma natural formando parte del complejo de **especies asociadas a las zonas agrícolas**. La situación más común en el Control Biológico es la combinación de distintos enemigos naturales para garantizar la reducción de las poblaciones plaga, por lo que es de gran importancia que estas especies sean capaces de reconocerse químicamente a fin de que sus poblaciones se segreguen en el cultivo, minimizando la competencia intragremial y ejerciendo así un control más eficaz.

- Tras haber obtenido en nuestros estudios información relevante acerca de la biología del sírfido y de algunas de sus interacciones con otros agentes de su sistema trófico en condiciones naturales, nos centramos en el estudio de la compatibilidad de este agente de control con otras estrategias comúnmente incluidas en los planes de control biológico e integrado de los cultivos bajo abrigo, abordando este estudio desde perspectivas novedosas y poco estudiadas hasta el momento:

Las estrategias de **control biológico por conservación** son muy interesantes tanto para conservar y potenciar las poblaciones naturales de sírfidos asociados a las áreas agrícolas como para optimizar sus sueltas dentro de los planes de control biológico aumentativo. Centrándose en el uso de **recursos florales** adicionales en el cultivo, es de gran importancia la adecuada selección de las especies de flor que van a utilizarse, pues se ha demostrado que el uso de especies inadecuadas, puede ocasionar problemas secundarios como favorecer el desarrollo de otras plagas o enfermedades o comportarse como malas hierbas, compitiendo así con el cultivo. Nuestro objetivo ha sido el estudio de la idoneidad y selección de varios recursos florales desde un doble punto de vista: su potencial atractivo, que favorecerá el establecimiento y permanencia de los sírfidos en los invernaderos y su efecto en la eficacia biológica de estos depredadores.

Hemos advertido que aunque todas las especies de plantas con flor estudiadas permiten a los sírfidos adultos madurar sexualmente y engendrar con éxito una primera generación, la alimentación con cada una de estas plantas favorece distintos componentes del *fitness* de estos depredadores. Nuestros resultados señalan que la **combinación** de más de un recurso floral puede ser la clave para optimizar los efectos positivos del manejo del hábitat en el control biológico. Hasta el momento se conocía el interés de combinar especies con distinta fenología de floración para asegurar la disponibilidad de recursos durante todo el ciclo de cultivo. Ahora además sabemos que el uso combinado de distintas especies florales puede maximizar la eficacia biológica de los sírfidos y probablemente la de otros enemigos naturales que dependan de las flores en alguna etapa de su ciclo biológico, potenciando así su acción como agentes de control.

De las plantas evaluadas, la flor del mastuerzo marítimo (*Lobularia marítima*) es la preferentemente visitada por los sírfidos adultos, y la alimentación exclusiva con esta especie durante la fase de adulto, ocasiona que la supervivencia de huevo a adulto y el tamaño corporal de los adultos de la siguiente generación sea mayor, indicando la capacidad de este sírfido para elegir aquellos recursos tróficos que incrementan en mayor medida el ***fitness de la futura generación***. Esta es la primera evidencia de un efecto materno o transgeneracional en un enemigo natural de pulgones relacionado con la dieta, y que demuestra además que el control por conservación ofrece un amplio campo de investigación con grandes expectativas. Basado en nuestros resultados, recomendamos la selección de recursos florales basada en estudios específicos para cada enemigo natural y dirigidos no sólo a la atracción del insecto, sino también a mejorar su eficacia biológica, para obtener los mejores resultados con el control biológico por conservación.

Adicionalmente, hemos demostrado que los sírfidos pueden madurar y engendrar nuevas generaciones alimentándose exclusivamente de las flores del cultivo, en este caso pimiento, destacando la importancia que tiene el propio cultivo en el mantenimiento de las poblaciones de enemigos naturales en los agroecosistemas, un campo que hasta el momento ha sido poco explorado dentro del control por conservación.

En cuanto a las estrategias combinadas de **control cultural** y biológico en este tipo de cultivos, se ha evaluado la compatibilidad de las **mallas fotoselectivas absorbentes de luz UV** con las sueltas de sírfidos, siendo este estudio uno de los primeros que aborda la compatibilidad entre esta estrategia y un depredador de pulgón. La efectividad de estas cubiertas se basa en el bloqueo de parte de la porción ultravioleta del espectro solar, para disminuir la entrada de insectos plaga a los invernaderos y su dispersión dentro de los mismos, aunque este empobrecimiento en UV puede también afectar a los agentes de control que se liberan en el invernadero, y es por ello que su efecto en los enemigos naturales, en este caso los sírfidos, también necesita ser evaluado.

El resultado obtenido es que no hay evidencias de que la actividad de los sírfidos adultos y de las fases inmaduras esté afectada negativamente de forma directa por el uso de estas mallas. Los resultados no son concluyentes acerca de si la capacidad de búsqueda de los lugares de oviposición y recursos florales en los sírfidos se ve perturbada por las mallas fotoselectivas, pues estos insectos pueden guiarse mediante estímulos químicos además de los visuales para su búsqueda. En cualquier caso, concluimos que **las mallas fotoselectivas absorbentes de radiación UV y las sueltas de sírfidos son estrategias compatibles** para su combinación en los planes de control integrado contra pulgones.

Esta tesis tiene tres aspectos clave que justifican su interés.

1) aporta información inédita sobre los requerimientos ambientales, la capacidad depredadora y las interacciones químicas con otros agentes de control del sírfido *S. rueppellii*. Esta información es valiosa y necesaria para llevar a cabo con éxito la cría masiva de este sírfido, así como para conocer cuál es el protocolo de actuación y el momento más adecuado para su adecuada liberación en campo.

2) Se han obtenido nuevas evidencias de la eficacia de una práctica ya recomendada para sírfidos y otros enemigos naturales como es la provisión de recursos florales en el cultivo, aportando información inédita sobre el efecto de la alimentación en el *fitness* de los insectos a través de varias generaciones.

3) aporta información novedosa sobre la compatibilidad del uso de los sírfidos con una estrategia de control cultural de uso creciente como son las mallas fotoselectivas, y para la cual no existía ningún estudio previo sobre su compatibilidad con agentes biológicos depredadores para el manejo integrado de plagas de pulgón.

4) Se pone de manifiesto la importancia de utilizar especies nativas de agentes de control en cultivos de invernadero del área mediterránea, habiéndose probado por vez primera en sírfidos su mejor adaptación a las particulares condiciones bióticas y abióticas de esta región.

Conclusiones

- 1.** *Sphaerophoria rueppellii* está adaptada a un amplio rango de temperaturas, (20°C-30°C) similares a las que se alcanzan en los invernaderos mediterráneos a lo largo del ciclo de cultivo. No sólo completa su ciclo a temperaturas elevadas, sino que además lo hace más rápidamente que a temperaturas moderadas.
- 2.** La humedad relativa es un factor clave en el desarrollo de las fases inmaduras de *S. rueppellii*, necesitando humedades ambientales superiores al 60% para desarrollarse satisfactoriamente.
- 3.** *Sphaerophoria rueppellii* presenta una voracidad similar, aunque ligeramente inferior, a *Episyrphus balteatus*, la única especie de sírfido comercialmente disponible por el momento.
- 4.** Las larvas de *S. rueppellii* pueden reducir el número de pulgones que consumen en condiciones de escasez de presas, resultando en un mayor tiempo de desarrollo y un menor tamaño corporal en la fase adulta, pero sin que incremente su tasa de mortalidad.
- 5.** Los compuestos volátiles tienen un papel clave en el comportamiento de oviposición de las hembras de *S. rueppellii*, pues estas son capaces de responder ante la presencia de plantas infestadas con pulgón, e incluso a la presencia de competidores de su especie en estas plantas, guiadas solo por estímulos olfativos.
- 6.** Los compuestos volátiles no presentan un papel importante en la detección de las colonias de pulgón durante la fase larvaria, al menos durante los dos últimos estadios.

- 7.** Las hembras de *S. rueppellii* evitan ovipositar en colonias de pulgón en presencia del coccinélido *Adalia bipunctata* cuando son capaces de ver y tocar las plantas, pero no muestran este comportamiento de evasión cuando únicamente se guían por semioquímicos volátiles.
- 8.** La alimentación con tres plantas con flor distintas favorece diferentes componentes del *fitness* de *S. rueppellii*. El cilantro aumenta en mayor medida los parámetros reproductivos (fecundidad y fertilidad), el mastuerzo marítimo incrementa el número de individuos de la primera generación que llegan a adultos, y su tamaño corporal. Las flores de pimiento reducen el tiempo de desarrollo de las larvas de la segunda generación.
- 9.** Las flores de mastuerzo marítimo son las visitadas preferentemente por sírfidos adultos de ambos sexos. Esta flor es la que aumenta en mayor medida el *fitness* de la primera generación, existiendo una relación entre la preferencia floral y el *fitness* de las futuras generaciones.
- 10.** Las mallas fotoselectivas absorbentes de radiación UV y las sueltas de sírfidos son estrategias compatibles para su combinación en los planes de control integrado contra pulgones. El uso de estas cubiertas no altera la búsqueda de recursos florales ni los parámetros reproductivos de los sírfidos adultos. Los estadios inmaduros tampoco se ven afectados negativamente, y su abundancia y dispersión en el invernadero se relaciona con la de su presa.

Apéndice

Documentos científicos y de difusión

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Imágenes



Imagen 1: Adulto de *Sphaerophoria rueppellii*



Imagen 2: huevo de *S. rueppellii*



Imagen 3: larva de *S. rueppellii* depredando un pulgón



Imagen 4: pupa de *S. rueppellii*

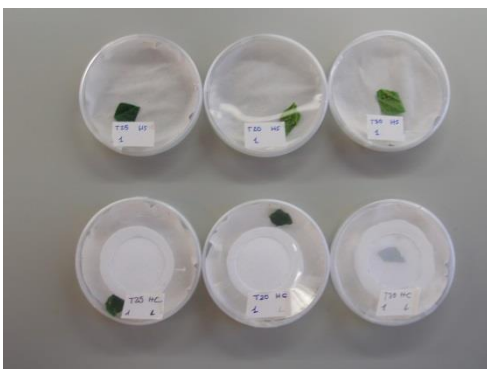


Imagen 5: placas de Petri normales y con malla en la tapa superior

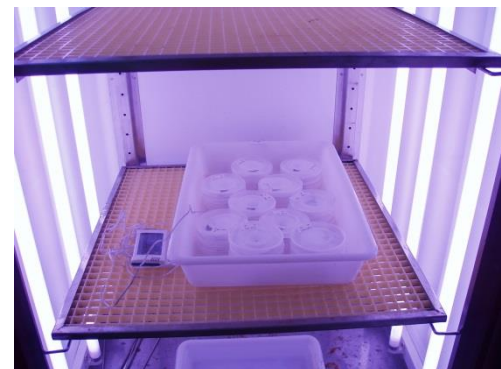


Imagen 6: cámara de cultivo FITOTRON



Imagen 7: flor de pimienta



Imagen 8: Inflorescencia de mastuerzo marítimo



Imagen 9: Inflorescencia de cilantro



Imagen 10: Invernaderos experimentales de la universidad de Alicante



Imagen 11: Olfatómetro de seis brazos laboratorio FARCE



Imagen 12: cámara central y brazos del olfactómetro



Imagen 13: Invernadero tipo túnel en estación experimental La Poveda



Imagen 14: módulo del invernadero con cultivo de nabo

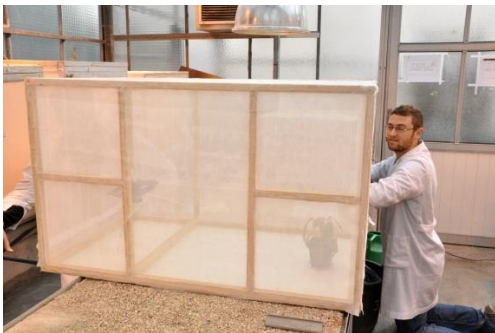


Imagen 15: Jaulones con malla Optinet



Imagen 16: Jaulones con malla Bionet y estándar bajo focos de espectro solar

Reunido el Tribunal que suscribe en el día de la fecha acordó otorgar, por a
la Tesis Doctoral de D. Rocco Amorós Jiménez la calificación de .

Alicante de de

El Secretario,

El Presidente,

UNIVERSIDAD DE ALICANTE

CEDIP

La presente Tesis de D. Rocco Amorós Jiménez ha sido registrada con el nº _____ del
registro de entrada correspondiente.

Alicante __ de _____ de ____

El Encargado del Registro,



Universitat d'Alacant
Universidad de Alicante



INTERACCIONES INSECTO-PATÓGENO-PLANTA
Y SUS AGENTES DE BIOCONTROL