Habitat management and the use of plant-based resources for conservation biological control

Gestión del hábitat y papel de los recursos vegetales en el control biológico por conservación

Lorena Pumariño Romero

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Memoria presentada por **Lorena Pumariño Romero** para
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Illustrations: Clara Herreros

Front cover: *Orius* spp. feeding on an alyssum flower

Back cover: Alyssum, buckwheat, phacelia and chamomile flowers. Faba bean and green bean stems.

It matters not how strait the gate,
How charged with punishments the scroll,
I am the master of my fate,
I am the captain of my soul.

William Ernest Henley (and some years later, Nelson Mandela)

A mi madre, mi padre y mi hermano

A la memoria de mi yaya

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Report of the supervisor of the Ph.D. Thesis in reference to its derived publications and the student's contribution to them

Dr. Oscar Alomar Kurz, Researcher of Sustainable Plant Protection Entomology (Institut de Recerca i Tecnologia Agroalimentàries), as supervisor of the Ph.D. Thesis authored by Ms. Lorena Pumariño Romero, and entitled *Habitat management and the use of plant-based resources for conservation biological control* (Gestión del hábitat y papel de los recursos vegetales en el control biológico por conservación),

INFORMS

That the results and conclusions achieved in the research developed by Ms. Lorena Pumariño Romero as part of her PhD Thesis have been organized in 6 chapters, which correspond to 6 papers (2 published, 1 accepted with revisions, 2 already submitted and 1 to be submitted). I attach the list of papers and manuscripts, together with the impact factor of each journal (2010 Journal Citation Reports, ISI Web of Knowledge).

1. Pumariño, L., O. Alomar and N. Agustí. 2011. Development of specific ITS markers for plant DNA identification within herbivorous insects. **Bulletin of Entomological Research** 101, 271 - 276. 2010 Journal Impact factor = **1.909**, in Quartile 1 of the 'Entomology' group (Ranks 13 out of 83).
2. Pumariño, L., O. Alomar, J. G. Lundgren. 2011. The influence of intraguild competitors on reproductive decisions by two predatory Heteroptera, *Orius insidiosus* (Anthocoridae) and *Nabis americoferus* (Nabidae). **Biocontrol Science and Technology** 21(11), 1321 - 1330. 2010 Journal Impact factor = **0.882**, in Quartile 3 of the 'Entomology' group (Ranks 43 out of 83).
3. Pumariño, L., O. Alomar (accepted pending revisions). The role of omnivory in the conservation of predators: *Orius majusculus* (Heteroptera: Anthocoridae) on sweet alyssum. **Biological Control**. 2010 Journal Impact factor = **2.164**, in Quartile 1 of the 'Entomology' group (Ranks 11 out of 83).
4. Pumariño, L., O. Alomar (submitted). *Lobularia maritima* as an insectary plant for the conservation of *Orius* spp.: biological control of *Frankliniella occidentalis*. **BioControl**. 2010 Journal Impact factor = **2.191**, in Quartile 1 of the 'Entomology' group (Ranks 10 out of 83).

5. Pumariño, L., O. Alomar, J.G. Lundgren (submitted). Diversity of floral and extrafloral nutritional resources on the fitness of an omnivorous bug, *Orius insidiosus* (Hemiptera: Anthocoridae). **Journal of Insect Conservation**. 2010 Journal Impact factor = **1.769**, in Quartile 1 of the 'Entomology' group (Ranks 17 out of 83).
6. Pumariño, L., D. Malcolm, M.A. JacomettiI, S.D. Wratten (submitted). Enhancement of vineyard ecosystem services by under-vine mulches. **International Journal of Agricultural Sustainability**. 2010 Journal Impact factor = **1.211**, in Quartile 1 of the 'Agriculture, Multidisciplinary' group (Ranks 12 out of 55).

and CERTIFIES

that the contribution of Ms. Lorena Pumariño has been very active as demonstrated by her first co-authoring of all manuscripts that conform this Ph.D. Thesis. Concretely, her participation included the following tasks(*):

- Definition of the objectives and focus of the research and the derived manuscripts
- Experimental design and laboratory and field work
- Preparation of the experimental material, sampling, counting, and identification
- Compilation of the results, data analysis and interpretation
- Main writing of the manuscripts, design and preparation of tables and figures, and responsible for the submission.

(*) The 6th chapter of the Thesis was carried out in New Zealand, as a consequence she could only participate in the laboratory and field work during 5 of the15 months of the research. In addition she participated in the definition of the objectives and focus of the research, experimental design, compilation of the results, data analysis and interpretation and main writing of the manuscript.

Finally, I certify that none of the co-authors of the manuscripts detailed above has used nor is going to use, implicitly or explicitly, the information produced and presented here with the purpose of elaborating another Ph.D. Thesis.

Barcelona, 20th January 2012
Dr. Oscar Alomar Kurz

Resumen

Introducción general

Control biológico por conservación y gestión del hábitat

Durante el siglo XX la agricultura se ha centrado en sistemas de producción muy intensivos y en gran medida dependientes de insumos externos como los fertilizantes o los plaguicidas. El uso de estas prácticas ha llevado a una simplificación del paisaje, siendo el monocultivo el mayor representante de la agricultura actual. Muchos autores han señalado que dicha pérdida de diversidad en los cultivos, además de generar una uniformidad del paisaje, ha disminuido la abundancia de enemigos naturales de las plagas debido a la supresión tanto de sus recursos alimenticios como de sus refugios (Andow, 1991; Tscharntke *et al.*, 2007). No obstante, en los últimos años ha habido un interés creciente en el uso de técnicas más respetuosas con el medio ambiente en la agricultura (Gurr *et al.*, 2004).

El Control Integrado de Plagas (CIP) surge en la agricultura actual como alternativa al excesivo uso de productos químicos. Concretamente, el CIP engloba el uso de medidas químicas, biológicas y culturales así como el uso de biotecnología (Eilenberg *et al.*, 2001). Dentro del CIP tiene gran importancia el control biológico de plagas, que consiste en el uso de enemigos naturales para mantener el nivel de plaga a niveles más bajos de lo que estarían en ausencia de los enemigos naturales (Debach, 1974). De acuerdo con Eilenberg *et al.* (2001) hay cuatro clases de control biológico: (1) Clásico, basado en la introducción intencionada de un agente de control exótico, (2) Inoculativo, consistente en la introducción de un agente de control biológico para que aumente su población y controle la plaga durante un periodo de tiempo largo, no permanente, (3) Inundativo, basado en la liberación de un agente de control biológico que permita suprimir una plaga únicamente con los organismos liberados y (4) por Conservación, que implica la modificación del ambiente o de las prácticas ya existentes para proteger y aumentar las poblaciones de enemigos naturales y de esa manera reducir los efectos de las plagas.

El Control Biológico por Conservación (CBC) implica la manipulación del entorno para aumentar la efectividad de los enemigos naturales, lo cual puede lograrse mediante un aumento de los factores positivosr así como una disminución de los factores negativos que afectan a los enemigos naturales (Landis *et al.*, 2000). El CBC se basa fundamentalmente en el uso de enemigos naturales autóctonos, dado que estos son capaces de colonizar los cultivos cuando se les necesita

(Barbosa, 1998; Pickett y Bugg, 1998), aunque esta técnica también puede ser útil para la conservación de enemigos naturales introducidos no autóctonos (Gurr y Wratten, 1999). Por otra parte, en el CBC el impacto de los enemigos naturales se potencia debido a que la mortalidad de estos es menor al disminuir el uso de plaguicidas (Gurr *et al.*, 2000). Al aplicar el CBC se deben tener en cuenta los siguientes factores que afectan a los enemigos naturales (Heinz *et al.*, 2004): medio ambiente (temperatura, fotoperiodo y ventilación), plaguicidas, acceso a fuentes de alimento alternativas, efectos de las plantas (lugares de oviposición o refugios) e interacciones con otros enemigos naturales. Debido a la influencia positiva del CBC en la conservación de los enemigos naturales, durante los últimos años ha habido un interés creciente en la aplicación del mismo (Jonsson *et al.*, 2010; Perdikis *et al.*, 2011).

Actualmente los sistemas agrarios presentan unas condiciones ambientales muy negativas para el correcto desarrollo de artrópodos beneficiosos y por tanto es vital la creación o incorporación en el medio de infraestructuras que favorezcan la conservación de dichos artrópodos (Landis *et al.*, 2000). La gestión del hábitat es una parte del CBC que consiste en la incorporación al medio de plantas que proporcionen a los enemigos naturales los recursos necesarios, como son alimentos vegetales (polen o néctar), presas o huéspedes alternativos o refugios (Landis *et al.*, 2000; Fiedler *et al.*, 2008). Hay distintos tipos de manejo del hábitat que pueden aumentar las poblaciones de artrópodos beneficiosos en los cultivos, ejemplos de esto son los "*beetle banks*", hileras de plantas refugio, cultivos intercalados y cubiertas o mantillos vegetales entre otros (Landis *et al.*, 2000 y referencias en artículo; Thomson y Hoffmann, 2007). De todos ellos, el uso de plantas insectario ha recibido gran atención en los últimos años (Hogg *et al.*, 2011; Nafziger y Fadamiro, 2011).

Enemigos naturales y plantas en los agroecosistemas

Las plantas representan todo un universo para los artrópodos, obteniendo de ellas no sólo alimento, sino también lugares apropiados para la oviposición, la cópula o como refugio. Por ello, dentro del CBC, las plantas son una herramienta imprescindible para el mantenimiento de los enemigos naturales en el cultivo. En los sistemas agrarios, un aumento de biodiversidad en lo que a especies de plantas se refiere, se relaciona con un aumento en la actuación de enemigos naturales, y por tanto con una reducción del efecto de las plagas (Andow, 1991). En consecuencia, es importante identificar y proporcionar la biodiversidad adecuada (Gurr *et al.*, 2005), ya que en ocasiones la introducción de plantas puede beneficiar a otros artrópodos además de a los enemigos naturales objeto de estudio (Araj *et al.*, 2009; Winkler *et al.*, 2009). Por tanto, se deben seleccionar las plantas que más convengan en cada caso concreto.

Existe un número elevado de estudios de campo que han demostrado que la presencia de plantas en flor aumenta la abundancia de enemigos naturales (Alomar *et al.*, 2006; Fiedler y Landis, 2007; Hogg *et al.*, 2011), así como un incremento del éxito del control de plagas (Irvin y Hoddle, 2007; Alomar *et al.*, 2008; Pease y Zalom, 2010). Además de la atracción que dichas plantas ejercen sobre los enemigos naturales, es bien conocido que estos usan los distintos componentes de las plantas (polen, néctar floral o extrafloral o la propia savia) como alimento (Alomar y Wiedenmann, 1996; Wäckers, 2005). Este alimento vegetal puede ser utilizado como un complemento a la dieta carnívora, o como un alimento imprescindible para su supervivencia durante periodos de escasez de presa (Lundgren, 2009). Existen cantidad de trabajos que han mostrado un aumento en la reproducción y supervivencia de depredadores y parasitoides como resultado del acceso a dichos recursos vegetales (Taylor y Pfannenstiel, 2009; Lundgren *et al.*, 2010; Nafziger y Fadamiro, 2011).

Sin embargo, a pesar del posible impacto positivo de las plantas sobre los artrópodos beneficiales, la incorporación de estas en los sistemas agrarios podría tener también efectos negativos (Huang *et al.*, 2011). De acuerdo con Gurr *et al.* (2005) hay varios riesgos asociados al hecho de la introducción de plantas en los ecosistemas agrarios que deberían ser evaluados antes de su introducción, como son por ejemplo, la introducción de plantas exóticas que puedan acabar convirtiéndose en invasivas, la aparición de depredación intragremial o competición asociada al aumento de densidad de enemigos naturales o el aumento de la presencia de otras especies a parte de la especie objeto de estudio. En ocasiones algunos herbívoros pueden beneficiarse de la introducción de dichas plantas ya que se alimentan de ellas (Baggen y Gurr, 1998; Hulshof *et al.*, 2003; Winkler *et al.*, 2009), o bien es el enemigo natural el que se alimenta de la planta, con un detrimento de la depredación o el parasitismo esperado (Skirvin *et al.*, 2007; Robinson *et al.*, 2008). A veces, son otros enemigos naturales, como los hiperparasitoides o depredadores intragremiales, los que se benefician de la adición de plantas, causando daños a la población del enemigo natural bajo estudio, y por tanto provocando una disminución del control de plagas (Araj *et al.*, 2009). La introducción de plantas en los sistemas agrarios puede, por tanto, ser perjudicial para el control biológico si no se tienen en cuenta las precauciones pertinentes. A pesar del riesgo que dicha introducción entraña, en general este ha sido un tema olvidado sobre el cual no se ha investigado lo suficiente. Sólo aquellas plantas seleccionadas que beneficien a los enemigos naturales sin incrementar el riesgo para el cultivo deberían introducirse en los sistemas agrarios

(Baggen y Gurr, 1998). Además, es importante conocer hasta que punto una planta insectario que puede alojar poblaciones plaga representa un riesgo para el cultivo, ya que si los enemigos naturales existentes en dicha planta son capaces de controlar las plagas, el riesgo podría ser descartado. Por tanto, estas interacciones entre plagas y enemigos naturales en cada especie vegetal usada como planta insectario se deberían estudiar en detalle antes de realizar una introducción en el sistema.

Los artrópodos realizan sus puestas fundamentalmente en las plantas, y por tanto las decisiones en cuanto a la oviposición están altamente influenciadas por las características de las propias plantas. Algunos insectos muestran preferencias de oviposición muy claras entre las distintas especies vegetales (Coll, 1996; Sanchez *et al.*, 2004; Lundgren y Fergen, 2006), o incluso entre distintos tejidos vegetales dentro de una misma planta (Sigsgaard, 2004; Groenteman *et al.*, 2006; Lundgren *et al.*, 2008). Adicionalmente, hay otros factores que pueden influir en las decisiones de oviposición, como pueden ser la presencia de otros enemigos naturales y las posibles interacciones que pueden tener lugar entre ambas especies, como ocurre en la depredación intragremial (Faraji *et al.*, 2001, 2002; Montserrat *et al.*, 2007). Recientemente se ha discutido el hecho de que las especies de depredadores puedan incluso evitarse mutuamente (Lundgren, 2011). Las plantas son escenarios muy complejos para los artrópodos dónde pueden tener lugar múltiples interacciones entre ellos y por tanto dichas interacciones deberían estudiarse en detalle.

Influencia de la fitofagia en el desarrollo de depredadores omnívoros

Existen muchos trabajos en la bibliografía que han documentado la importancia del alimento vegetal para los parasitoides (Berdnt y Wratten, 2005; Begum *et al.*, 2006; Lee y Heimpel, 2008). Sin embargo el estudio de los beneficios de dicho alimento vegetal para los depredadores es relativamente reciente, observándose un creciente interés en el estudio de los beneficios del alimento vegetal para los depredadores omnívoros, los cuales se alimentan tanto de presa como de planta (Coll y Guerson, 2002; Lundgren, 2009). Algunos de estos depredadores omnívoros se caracterizan porque consumen presa o planta durante distintos estadios de su vida (*life-history omnivores*) (Coll y Guerson, 2002), mientras que otros consumen la presa y la planta a lo largo de toda su vida (*lifelong omnivores*) (Eubanks y Styrsky, 2005). A pesar de dichas diferencias, la biología y el desarrollo de ambos pueden verse muy influenciados por la especie vegetal y sus características concretas.

Se ha estudiado la influencia del alimento vegetal en el desarrollo de las ninfas (Cottrell y Yeargan, 1998; Eubanks y Denno, 1999), la reproducción y la supervivencia de adultos (Limburg y Rosenheim, 2001; Beckman y Hurd, 2003), la supervivencia de depredadores en ausencia de presa (Eubanks y Denno, 1999; Leon-Beck y Coll, 2007), así como su influencia en el control de plagas, tanto positiva (Gillespie y McGregor, 2000) como negativamente (Robinson *et al.*, 2008). Sobre todas las características de los depredadores omnívoros, hay una que presenta gran interés, y es que sus hábitos alimenticios vegetarianos les permiten alimentarse de la planta y por tanto sobrevivir durante los tiempos de ausencia o baja densidad de presa (Eubanks y Denno, 1999; Leon-Beck y Coll, 2007). En dichas circunstancias, las posibilidades de permanencia de los mismos en el sistema agrario aumentan, ya que disminuyen el riesgo de migración a otros lugares en busca de alimento. Sin embargo, en una situación donde no exista presa o algún recurso vegetal alternativo, la probabilidad de migración aumenta considerablemente. Por lo tanto es muy interesante la conservación de estos depredadores omnívoros en los cultivos o en los alrededores (lo cual se consigue con la inclusión de plantas insectario), ya que pueden mantenerse con niveles de plaga muy bajos y son capaces de frenar cualquier aumento no deseado de plaga (Coll and Guerson, 2002).

Existen varios trabajos publicados sobre depredadores omnívoros pertenecientes a distintas familias que se alimentan de savia (Stoner, 1972; Ruberson *et al.*, 1986; Lundgren *et al.*, 2008) o el polen (Corey *et al.*, 1998; Broufas y Koveos, 2000; Beckman y Hurd, 2003), sin embargo, la importancia del néctar como alimento vegetal para estos depredadores así como el efecto en el desarrollo de los mismos, no se ha empezado a estudiar en detalle hasta hace relativamente poco tiempo (Limburg y Rosenheim, 2001; Lundgren y Seagraves, 2011; Portillo *et al.*, 2012). A pesar de que las plantas son fuentes alimenticias muy importantes para estos depredadores omnívoros, los beneficios que el polen y el néctar floral o extrafloral representan para ellos son todavía un fenómeno poco estudiado.

Biología molecular aplicada al control biológico por conservación

Los paisajes agrícolas compuestos principalmente por cultivos anuales son constantemente iniciados e interrumpidos y representan un gran problema para el establecimiento de enemigos naturales debido a su naturaleza efímera (Alomar y Albajes, 2003). En dichas situaciones los artrópodos se encuentran con la ausencia no sólo de comida, sino también de refugio y de lugares para ovipositar. En consecuencia, esta destrucción periódica tanto de los cultivos como de las plantas adventicias circundantes hace difícil la permanencia de depredadores y parasitoides, que deben recolonizar los campos constantemente (Alomar y Albajes, 2003; Alomar *et al.*, 2006). Dicha situación provoca la disminución en el control de las poblaciones de plaga en momentos críticos como puede ser el inicio del cultivo (Landis, 2000). En estos lugares cobra especial relevancia la conservación de enemigos naturales por medio de la gestión del hábitat, ya que es posible mantener en el entorno depredadores y parasitoides autóctonos que controlen las poblaciones plaga (Alomar y Albajes, 2003; Alomar *et al.*, 2006). Por tanto es muy importante la identificación de las plantas insectario más apropiadas que sirvan para mantener dichas poblaciones de enemigos naturales cerca de los cultivos.

Si bien dichas plantas insectario alojan poblaciones elevadas de enemigos naturales, estos pueden provenir también de otros lugares circundantes. Dichas migraciones aumentan el éxito de los programas de control biológico pero no permiten evaluar correctamente los beneficios que supone una planta insectario concreta para los enemigos naturales, ni permiten la identificación de otros posibles refugios, ya que los artrópodos pueden migrar desde otras fuentes cercanas. (Corbett y Rosenheim, 1996). Además, no siempre es posible conocer el origen de los depredadores que colonizan los cultivos, porque pueden no observarse refugios conocidos de dichos depredadores en el entorno (Alomar *et al.*, 2002; Gabarra *et al.*, 2004). Se han evaluado distintas técnicas de marcado y seguimiento en artrópodos para estudiar el movimiento y dispersión de los mismos entre los cultivos y las áreas aledañas (Lavandero *et al.*, 2004; Scarratt *et al.*, 2008; Stephens *et al.*, 2008). Sin embargo todos estos métodos presentan dificultades a la hora de ser usados en estudios de campo, ya que o bien la planta o bien el artrópodo deben ser marcados de antemano. Otros métodos consisten en el estudio del contenido estomacal de los artrópodos mediante técnicas serológicas, identificaciones morfológicas o cromatografía (Heimpel *et al.*, 2004; Silberbauer *et al.*, 2004; Jones *et al.*, 2006). Por su parte estos últimos métodos presentan el inconveniente de que son muy laboriosos y necesitan de mucho tiempo, lo cual no sería aplicable a estudios de campo de gran envergadura.

Un método alternativo para estudiar la dispersión de los depredadores omnívoros desde sus refugios hasta los cultivos cercanos podría ser la identificación del ADN de planta ingerido por el artrópodo de manera similar a como se identifica la presa mediante la técnica PCR (*Polimerase Chain Reaction*). Desde los primeros estudios realizados sobre detección de presa ingerida a finales de los noventa (Agusti *et al.*, 1999; Agustí *et al.*, 2000), han ido publicándose gran número de trabajos hasta convertirse en un tema de estudio muy común. Sin embargo, la identificación de ADN de planta en el digestivo de artrópodos es un tema más bien reciente que ha ido cobrando importancia durante los últimos años (Miller *et al.*, 2006; Matheson *et al.*, 2007; Jurado-Rivera *et al.*, 2009). En dichos estudios los fragmentos vegetales fueron secuenciados y comparados con homólogos en la base de datos BLAST, lo cual es un método muy útil para la detección del material vegetal ingerido pero no demasiado práctico para estudios de campo, en los que se deben analizar un número de individuos muy elevado. El desarrollo de marcadores moleculares específicos de planta capaces de identificar el ADN de las plantas ingeridas dentro de los artrópodos sería por tanto un método mucho más apropiado y barato para el desarrollo de estudios de campo. Por otra parte, con este método sería posible le detección de parasitismo y depredación, así como la ingestión de restos vegetales al mismo tiempo, lo cual ayudaría a conocer hasta qué punto los enemigos naturales combinan la alimentación zoófaga y fitófaga en su dieta.

Control biológico por conservación y servicios ecosistémicos

El CBC forma parte de lo que se conoce como servicios ecosistémicos (Fiedler *et al.*, 2008). Dicho concepto fue definido por Daily (1997) como aquellos procesos mediante los cuales los ecosistemas mantienen y satisfacen las necesidades del ser humano. Algunos trabajos han demostrado como los sistemas agrarios tradicionales han mermado estos servicios ecosistémicos comparado con otros sistemas más sostenibles como es el caso de la agricultura ecológica (Sandhu *et al.*, 2008). Existen sin embargo algunas prácticas agronómicas, como es el uso de mantillos vegetales entre muchas otras, que pueden aumentar los servicios ecosistémicos en los cultivos y también mejorar notablemente el CBC por medio de una disminución de las poblaciones de artrópodos herbívoros, lo cual puede lograrse con el aumento de las poblaciones de enemigos naturales y por tanto el aumento de la depredación y parasitismo de las plagas.

El uso de mantillos vegetales proporciona múltiples servicios al ecosistema como son control de plantas adventicias (Elmore *et al.*, 1997; Brown y Tworkoski, 2004), mejora de la fertilidad del suelo (Agnew *et al.*, 2005) y la capacidad de infiltración (Buckerlield y Webster, 2001), reducción de las fluctuaciones de la temperatura (Mundy y Agnew, 2002), mejora de la formación del suelo por parte de lombrices de tierra (Buckerlield y Webster, 2001) e incluso una reducción en la incidencia de algunas enfermedades en los cultivos (Jacometti *et al.*, 2007a, b). En relación al CBC, el uso de mantillos vegetales puede también proporcionar beneficios en el control de plagas. Esta mejoría puede conseguirse mediante una disminución de las poblaciones de herbívoros así como mediante un aumento de las poblaciones de enemigos naturales en los mantillos, lo que puede conllevar un mayor control de plagas en los cultivos (Mathews *et al.*, 2002; Brown y Tworkoski, 2004; Suckling *et al.*, 2006; Thomson y Hoffmann, 2007). Estos múltiples beneficios que los mantillos vegetales aportan a los cultivos podrían evaluarse de manera conjunta e integrarse, y tanto el aumento en la diversidad de artrópodos beneficiosos como la mejora de los servicios al ecosistema podrían evaluarse de forma conjunta.

Especies objeto de estudio en la Tesis

La aplicación del CBC es especialmente útil en sistemas agrícolas muy intensivos dónde el hábitat es marcadamente inestable ya que, como se ha mencionado anteriormente, los cultivos se inician y terminan constantemente, lo cual hace que tanto la instalación como el mantenimiento de los enemigos naturales sea una tarea difícil. Como se ha explicado con anterioridad, una posible solución al problema sería proporcionar refugios a los artrópodos beneficiosos. En estos ambientes tan cambiantes e inestables toman mayor relevancia los depredadores omnívoros, dada su mayor capacidad para sobrevivir en el medio cuando el acceso a la presa es muy limitado o inexistente (Eubanks y Denno, 1999).

Un grupo muy importante de depredadores omnívoros son los miembros pertenecientes al orden Hemiptera (Alomar y Wiedenmann, 1996). En esta Tesis se seleccionaron depredadores Hemípteros pertenecientes a las familias Anthocoridae y Miridae, dada su relevancia en programas de CBC en cultivos tan importantes como la lechuga o el tomate. *Orius majusculus* (Reuter) y *O. laevigatus* (Fieber) (Hemiptera: Anthocoridae) son especies paleárticas que se encuentran de forma común en Europa (Riudavets, 1995), donde han sido identificados como depredadores autóctonos del introducido trips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Riudavets y Castane, 1998). Se ha observado que depredadores del género *Orius* spp. abundan de manera espontánea en el cultivo de lechuga, donde controlan de forma efectiva plagas como los trips o los pulgones (Arnó *et al.*, 2002), siendo por tanto su mantenimiento en dichos entornos muy aconsejable. Otro depredador omnívoro importante es *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae), el cual se encuentra ampliamente extendido a lo largo de la costa mediterránea (Goula, 1986), depredando gran cantidad de presas. Este depredador se ha usado de manera efectiva para controlar poblaciones de mosca blanca, siendo muy efectivo en programas de control biológico en tomate (Avilla *et al.*, 2004). Otro antocórido relevante es *Orius insidiosus* Say, importante depredador autóctono en América del Norte en cultivos extensivos como el maíz, la soja o la alfalfa, que depreda fundamentalmente trips y pulgones (Baez *et al.*, 2004; Rutledge y O'Neil, 2005; Seagraves y Yeargan, 2009). En algunos de estos cultivos, dicho depredador comparte el hábitat con otros depredadores como es el caso de *Nabis americoferus* Carayon (Hemiptera: Nabidae) (Coll y Ridgway, 1995; Clements y Yeargan, 1997), un depredador generalista en los cultivos del Medio Oeste de EEUU. La relevancia de dichos depredadores como agentes de control biológico en los sistemas agrarios los convierte en interesantes candidatos para programas de conservación, y por esta razón fueron seleccionados para esta Tesis.

La primera planta seleccionada para este trabajo y con la que se han desarrollado varios apartados de esta Tesis fue *Lobularia maritima* L. Uno de los motivos principales de su elección fue los buenos resultados observados en cuanto a establecimiento y mantenimiento de insectos del género *Orius*. spp en esta planta en periodos entre cultivos (Alomar *et al.*, 2008). Por otra parte, es una planta mediterránea que presenta un periodo de floración muy largo (Pico y Retana, 2000) y que ha sido usada con éxito en muchos programas de conservación de distintos enemigos naturales.

Objetivos

El objetivo general de esta Tesis es investigar hasta qué punto el uso de recursos vegetales puede contribuir al CBC mediante la gestión del hábitat. Para desarrollar este objetivo general, la Tesis se ha centrado en seis objetivos específicos, distribuidos en sendos capítulos:

(1) **Capítulo 1**. Determinar la capacidad de *L. maritima*, y el efecto de la presencia de presa en la conservación del depredador omnívoro *O. majusculus* y compararlo con un control, vainas de judía verde, así como estudiar los efectos del cambio de planta hospedante en su reproducción.

(2) **Capítulo 2**. Determinar la depredación de *Orius* spp. sobre poblaciones del trips *F. occidentalis* en *L. maritima* y como esta afecta a la reproducción de *O. majusculus*, así como determinar la dispersión de *O. majusculus* hacia un cultivo circundante.

(3) **Capítulo 3**. Determinar la influencia de los distintos niveles de acceso al néctar y polen de varias plantas a la supervivencia, reproducción y estado nutricional de *O. insidiosus*, así como conocer hasta qué punto este depredador se ve afectado por un aumento en la diversidad vegetal.

(4) **Capítulo 4**. Determinar las interacciones existentes entre *O. insidiosus* y otro depredador hemíptero, *N. americoferus*, así como estudiar el efecto de estas interacciones tanto en la intensidad como en el lugar de oviposición de cada depredador en presencia de la otra especie.

(5) **Capítulo 5**. Diseñar marcadores moleculares específicos que permitan la identificación de ADN de tomate una vez ingerido por insectos con distintos hábitos alimenticios (masticador y succionador) e identificar los periodos de detección del ADN de tomate en dichas especies, así como determinar la utilidad de dichos marcadores moleculares en estudios de campo.

(6) **Capítulo 6**. Determinar el potencial de la aplicación de mantillos vegetales para aumentar la biodiversidad de los invertebrados de suelo y mejorar los servicios ecosistémicos en el suelo en viñedo.

Resultados y discusión general

Los distintos experimentos realizados en esta Tesis han permitido profundizar en cómo los recursos vegetales pueden contribuir a la mejora de programas de control biológico por conservación en cultivos hortícolas, así como si dichos recursos vegetales pueden mejorar algunos servicios al ecosistema en viñas.

Trabajos previos han mostrado que el género *Orius* consigue establecerse y mantenerse en *L. maritima* durante los periodos de tiempo entre un cultivo y otro (Alomar *et al.*, 2008). Además esta planta es mediterránea y presenta un periodo de floración muy largo, características que hacen que sea una planta muy interesante para ser usada en programas de conservación, razón por la cual fue utilizada en gran parte de los experimentos de esta Tesis. En el **Capítulo 1** se demostró que, de hecho, *L. maritima* proporciona los recursos necesarios para la conservación de *O. majusculus*. El depredador sobrevivió en *L. maritima* sin presa durante un periodo de tiempo similar al obtenido en el control (vainas de judía verde con presa), hecho que resalta los beneficios de usar una planta con polen y néctar respecto a una estructura tan simple como son las vainas de judía. Otros autores han reseñado los beneficios de usar flores sobre vainas de judía tanto para la reproducción como para la supervivencia de otro depredador del mismo género, *O. insidiosus* (Mendes *et al.*, 2005; Carvalho *et al.*, 2010). Por otra parte, aunque *O. majusculus* fue capaz de reproducirse sobre *L. maritima* cuando no disponía de presa, el máximo reproductivo sobre la planta se consiguió cuando se añadió presa al sistema. Con la adición de presa no sólo la fecundidad obtenida en *L. maritima* fue mucho más elevada que en el control (las vainas de judía), sino que además la oviposición se alargó durante un tiempo mucho mayor. Estos resultados se asimilan a los de otros trabajos donde se ha observado que tanto la fecundidad como la supervivencia de *Orius* spp. fueron mucho mayores cuando se añadió presa (Funao y Yoshiyasu, 1995; Cocuzza *et al.*, 1997). Adicionalmente, los resultados del experimento de cambio de planta huésped mostraron que la reproducción de *O. majusculus* fue similar en ambas plantas (*L. maritima* y vainas de judía verde), siempre que contaran con la presencia de presa, independientemente de la planta origen de los depredadores. Este último resultado enfatiza la importancia de suplementar las dietas de *O. majusculus* con presa para obtener la máxima reproducción posible del depredador. No obstante, el hecho de que la reproducción de *O. majusculus* en *L. maritima* no se vea influenciada por la planta origen en la que el depredador se crió es una característica interesante en el caso de que fueran necesarias sueltas comerciales de dicho depredador, ya que el hecho de usar *L. maritima* como planta insectario probablemente aseguraría el establecimiento del mismo. Los resultados obtenidos en este primer capítulo indican que *L. maritima* puede ser una planta insectario muy adecuada para la conservación de *O. majusculus* en los cultivos durante periodos de escasez de presa, o incluso en ausencia total de la misma.

El uso de recursos vegetales para conservar enemigos naturales en los cultivos o alrededor de ellos se considera una herramienta muy importante en los sistemas agrarios. Sin embargo, el mero hecho de aumentar la biodiversidad no siempre implica beneficios ya que en ocasiones puede entrañar también riesgos para los cultivos (Gurr *et al.*, 2005). Nuestros trabajos previos mostraron que *L. maritima* es capaz de mantener a *O. majusculus* y por tanto se puede considerar a esta planta como una buena candidata a ser usada en la conservación de *O. majusculus*. Sin embargo, es importante conocer también hasta qué punto esta planta puede promover la presencia de plagas en los cultivos, ya que hay trabajos que han documentado la coexistencia de *Orius* spp. con su presa principal, trips, en varias plantas adventicias (Silveira *et al.*, 2005; Atakan y Tunc, 2010). Por tanto, en el **Capítulo 2** de esta Tesis se investigó por un lado la depredación ejercida por parte de *Orius* spp. sobre trips en *L. maritima*, y por otro cómo dicha depredación afectaba a la reproducción de *O. majusculus*. Las dos especies de *Orius* usadas en esta Tesis, *O. majusculus* y *O. laevigatus,* fueron capaces de reducir las poblaciones de trips tanto en *L. maritima* como en el control (vainas de judía) en condiciones de laboratorio. Otros estudios han mostrado similares resultados de estas dos especies de *Orius* depredando trips de manera efectiva en vainas de judía (Riudavets y Castane, 1998; Tommasini *et al.*, 2004). Sin embargo, mientras que la capacidad de *O. majusculus* para reducir las poblaciones de trips no se vio afectada por la planta, la eficacia de *O. laevigatus* para reducir trips en *L. maritima* sí se vio reducida en relación a las vainas de judía. En consecuencia, *O. majusculus* representa una opción mejor para programas de CBC en los que se use *L. maritima* como planta insectario. En los ensayos realizados en el laboratorio durante un periodo de tiempo más largo, se confirmó que *L. maritima* es también un buen huésped para la reproducción de trips, donde este se reproduce satisfactoriamente. A pesar de ello las poblaciones de trips se vieron reducidas en presencia de *O. majusculus*. El depredador se reprodujo e instaló en *L. maritima* cuándo disponía de trips, aunque previamente había mostrado un mejor desarrollo en una dieta compuesta por huevos de *Ephestia kuehniella* (capítulo 1). Esta mejoría en la reproducción tanto de *O. majusculus* como de otros *Orius* spp. cuando disponen de huevos de *E. kuehniella* en lugar de trips ya se ha observado en otros estudios (Kiman y Yeargan, 1985; Tommasini *et al.*, 2004). Sin embargo esto no es extraño, ya que los huevos de lepidóptero suponen una dieta altamente nutritiva (Cohen, 1989). La depredación sobre trips permitió la reproducción de *O. majusculus* aunque no parece que esta dieta aporte más de lo que aporta *L. maritima* en sí misma. En condiciones de invernadero, el número de trips fue menor en los tratamiento con altas densidades de *O. majusculus* comparado con el tratamiento con bajas densidades, aunque no de forma significativa. Dichos resultados contrastan con los obtenidos por otros autores en los que *O. majusculus* redujo las poblaciones de trips en invernadero tanto en cultivos hortícolas cómo en ornamentales (Blaeser *et al.*, 2004; Fitzgerald, 2006). A pesar de ello, *O. majusculus* logró reproducirse de manera satisfactoria en *L. maritima* y además fue capaz de dispersarse al cultivo circundante, plantas de lechuga. Aunque se debería estudiar con mayor profundidad en condiciones de invernadero, parece que la presencia de *L. maritima* en los cultivos podría mejorar el establecimiento de *O. majusculus*, sin favorecer a las poblaciones de trips, y por tanto ser una buena candidata a conservar las poblaciones de *Orius* spp.

En el **Capítulo 3** se estudió cómo la utilización de distintas especies vegetales como huéspedes afecta al desarrollo de *O. insidiosus*, así como el efecto que tiene sobre esta especie un incremento en la diversidad vegetal. Específicamente se investigó cómo el uso de recursos vegetales como el néctar y el polen afectan a la supervivencia, reproducción y estado nutricional de *O. insidiosus*. Tres plantas, *L. maritima*, *Fagopyrum esculentum* y *Phacelia tanacetifolia* se seleccionaron para esta Tesis por ser de las plantas más citadas en la bibliografía en programas de CBC (Fiedler *et al.*, 2008). Otras dos especies se seleccionaron en base a su acceso a sus recursos, tanto florales como extra florales: *Vicia faba*, que posee nectarios extra florales y *Matricaria chamomilla*, cuyos nectarios florales se encuentran escondidos en una inflorescencia tipo capítulo (Patt *et al.*, 1997). Se observó que las distintas especies vegetales afectaron de maneras distintas a la supervivencia, a la reproducción y al estado nutricional de *O. insidiosus*. Sin embargo, el acceso a los recursos florales y extra florales sólo influyó en la supervivencia, pero no en la reproducción o el estado nutricional de *O. insidiosus*. De entre las especies usadas, *P. tanacetifolia* mejoró la supervivencia y reproducción del depredador mientras que los niveles de glicógeno fueron mayores en los individuos de *O. insidiosus* que se alimentaron en *V. faba*. El acceso a los recursos florales y extra florales dio lugar a una mayor supervivencia del depredador y a un porcentaje menor de depredadores desaparecidos, lo cual parece indicar que *O. insidiosus* no sólo se alimenta del floema vegetal, sino que consume también algunas de las otras partes vegetales, como el polen o el néctar. Mientras que las distintas especies vegetales parecieron tener tan sólo un efecto inmediato en la supervivencia de *O. insidiosus*, el acceso a los recursos florales y extra florales perduró de manera marginal en *O. insidiosus*, el cual sobrevivió ligeramente más tiempo cuando había estado confinado previamente en las plantas que poseían dichos recursos expuestos. Un aumento en la diversidad vegetal no generó variaciones en la supervivencia de *O. insidiosus*. Sin embargo, mientras que las longevidades en el primer experimento (donde el depredador se mantuvo en vainas de judía) fueron muy bajas, las hembras de *O. insidiosus* expuestas en este segundo experimento a plantas completas, sobrevivieron durante mucho más tiempo. Este resultado resalta nuevamente la importancia de recursos vegetales como el polen o el néctar comparado con una estructura más simple como las vainas de judía para *O. insidiosus*. De este modo, se obtuvieron resultados similares a los obtenidos en el Capítulo 1 para *O. majusculus*, lo que demuestra la importancia del polen y néctar para la conservación de *Orius* spp. La fecundidad obtenida para *O. insidiosus* fue muy distinta dependiendo de si las plantas se usaron de manera individual o conjunta. Mientras que *V. faba* fue el sustrato de oviposición en el que *O. insidiosus* puso el menor número de huevos cuando las plantas se ofrecieron de manera individual, dicha especie contó con el mayor número de huevos puestos por *O. insidiosus* cuando todas las plantas se ofrecieron de manera conjunta. Es decir, dichos resultados muestran que es probable que *O. insidiosus* considere a *V. faba* como un buen sustrato de oviposición, pero solamente cuando en el entorno hay disponibles otras fuentes de alimento más nutritivas para el desarrollo de su descendencia. Esto no sorprende, ya que algunos trabajos han demostrado que *O. insidiosus* prefiere ciertas plantas específicas como sustratos de oviposición (Coll, 1996; Lundgren y Fergen, 2006), o que incluso la especie vegetal es más importante para *O. insidiosus* que la presencia de presa a la hora de seleccionar dónde poner sus huevos (Seagraves y Lundgren, 2010). En resumen, estos resultados confirman que las plantas pueden otorgar beneficios muy variados para el desarrollo de *O. insidiosus*, pero que estos dependerán de diversos factores. Por tanto, es muy importante evaluar e identificar qué planta insectario es la más apropiada para la conservación de cada enemigo natural en cada programa concreto.

Las plantas representan un complejo hábitat para los insectos, en las cuales estos se alimentan, ponen sus huevos, se refugian e interaccionan con otros artrópodos. Con el fin de conocer las interacciones existentes entre depredadores pertenecientes al orden Hemiptera y de cómo dichas interacciones afectan a sus decisiones reproductivas, se estudió la reproducción de *O. insidiosus* y *N. americoferus* de manera individual y cuando coexistieron ambas especies. Concretamente, en el **Capítulo 4**, se estudió cómo dichos depredadores varían tanto la intensidad como el lugar de oviposición en la presencia de la otra especie. En soja, Clements y Yeargan (1997) mostraron que las dos especies se encontraban en diferentes partes de la planta, con lo cual la interacción entre ellos se esperaba mínima. En el presente estudio, por el contrario, se observó que estas dos especies de depredadores son capaces de percibir la presencia de la otra especie en plantas de judía. Ambos depredadores pusieron más huevos en presencia de la otra especie que cuando se expusieron a miembros de su especie. *Orius insidiosus* puso ligeramente más huevos en hojas y peciolos mientras que *N. americoferus* puso una cantidad de huevos significativamente mayor en peciolos y peciólulos. Cuando los depredadores sólo contaban con la presencia de conspecíficos, *O. insidiosus* realizó su puesta mayoritariamente en la parte superior de la planta mientras que *N. americoferus* puso sus huevos de manera equitativa a lo largo de toda la planta. Sin embargo, cuando las dos especies se encontraban presentes, *N. americoferus* puso un mayor número de huevos en el cuarto superior de la planta mientras que *O. insidiosus* puso marginalmente más huevos en una zona más baja. Estos resultados contrastan con el trabajo de (Isenhour y Yeargan, 1982) que mostraron que cuando *N. roseipennis* y *O. insidiosus* se encontraban juntos en soja, *O. insidiosus* puso un mayor número de huevos en la parte superior de las plantas, mientras que *N. roseipennis* prefirió ovipositar en la parte media. Los resultados de esta Tesis demuestran que *O. insidiosus* y *N. americoferus* pueden detectar la presencia de la otra especie y que sus decisiones reproductivas están muy posiblemente influenciadas por interacciones intragremiales.

En sistemas agrarios donde predomina el monocultivo, los insectos beneficiosos deben refugiarse en la vegetación circundante durante los periodos en los que no hay cultivo. Una vez el nuevo cultivo se siembra, estos insectos deben colonizarlo nuevamente desde sus refugios. La identificación de dichos refugios es por tanto muy importante para mantener a los enemigos naturales cerca de los campos de cultivo, y de esa manera favorecer dichas colonizaciones. La identificación de refugios no siempre es sencilla, debido en parte a que no existe una técnica que pueda usarse de manera fácil y rápida en estudios de campo a gran escala, en los que debe analizarse un número de insectos muy elevado. En esta Tesis se ha propuesto el desarrollo de marcadores moleculares de planta que permitan una rápida identificación de las especies vegetales en el digestivo de los insectos mediante PCR, usando el tomate como planta modelo. Concretamente en el **Capítulo 5** se ha comprobado que es posible detectar el ADN de tomate inmediatamente después de ser ingerido en todos los individuos que se evaluaron pertenecientes a tres especies distintas: *Macrolophus pygmaeus* (un depredador omnívoro con hábitos alimenticios succionadores), *Tuta absoluta* y *Heliothis armígera* (larvas herbívoras con hábito alimenticio masticador). El tiempo de digestión varió dependiendo del hábito alimentico de la especie, siendo la vida media del ADN de tomate en el digestivo mayor en los insectos masticadores que en el chupador. Estos resultados difieren de los obtenidos en otros estudios en los que los periodos de detección de presa fueron más largos en insectos chupadores que en masticadores (Greenstone *et* *al.*, 2007; Hosseini *et al.*, 2008). Por otra parte, también se detectó el ADN de tomate en insectos de campo, con porcentajes de detección más altos en ninfas y hembras que en machos. Cuando estos resultados se compararon con el trabajo de Moreno-Ripoll *et al.* (2009), donde se analizó la ingestión de presa en los mismos insectos utilizados en esta Tesis, se observó una detección simultánea de planta y presa dentro del mismo individuo, sobre todo en ninfas. Esta detección simultánea indica la existencia de dieta mixta y confirma un modelo en el que la alimentación vegetal es esencial para la depredación (Sinia *et al.*, 2004). Estos resultados demuestran que la utilización de marcadores moleculares específicos de planta permitiría la identificación de plantas en las cuales los insectos habrían estado alimentándose previamente. Esta información podría ser muy valiosa para entender la colonización por parte de enemigos naturales desde sus refugios a los cultivos, lo cual sería muy útil para la elección de posibles plantas insectario en programas de CBC.

Además de que los recursos vegetales pueden contribuir a la conservación de enemigos naturales, pueden presentar además otros beneficios añadidos, como son los servicios al ecosistema. En el **Capítulo 6** de esta Tesis se comparó la utilidad del uso de varios mantillos vegetales (restos de hierba segada, paja de lino y paja de guisante) con un control (suelo desnudo) para mejorar los servicios ecosistémicos en viña. Concretamente se evaluó el efecto de los mantillos vegetales en la biodiversidad de los invertebrados en el suelo, así como en la mejora de varios servicios al ecosistema en el suelo en dos viñedos con características bien distintas. En general, tanto el número de artrópodos como el de lombrices de tierra encontrado en ambos viñedos fue mayor en los lugares donde se habían usado los mantillos vegetales, algo similar a lo encontrado en estudios previos (Buckerfield y Webster, 1996; Brown y Tworkoski, 2004; Thomson y Hoffmann, 2007). La presencia de los mantillos redujo las fluctuaciones en las temperaturas, así como aumentó la humedad del suelo en uno de los viñedos, resultados comparables a otros trabajos (Mundy y Agnew, 2002). En ambos viñedos la depredación de *Epiphyas postvittana* (importante lepidóptero que causa numerosos daños en viña) en la parte aérea de las viñas fue similar en todos los tratamientos. Esto difiere de otros trabajos, donde el porcentaje de depredación en la parte aérea fue más elevado en los lugares en los que se habían usado mantillos vegetales (Mathews *et al.*, 2002; Brown y Tworkoski, 2004). Una explicación podría ser el hecho de que el experimento de esta Tesis se desarrollara en otoño, momento en el que los insectos comienzan a entrar en diapausa y su activad se va ralentizando. Los parámetros evaluados durante la cosecha sí se vieron afectados por los distintos tratamientos y variaron de un viñedo al otro. Sin embargo, y a pesar de dichas variaciones, en general los mantillos vegetales mejoraron los parámetros en relación al suelo desnudo.

En general puede concluirse que los resultados obtenidos en esta Tesis han demostrado que el uso de varios recursos vegetales puede mejorar de manera notable la aplicación de los programas de CBC, así como de servicios al ecosistema en los cultivos, aunque las especies vegetales deben seleccionarse con cuidado y según se adapten mejor a cada situación concreta.

Conclusiones

Conclusiones al **Capítulo 1**:

- El uso de *L. maritima* sin presa asegura la conservación y una mínima reproducción de *O. majusculus*. Sin embargo para lograr una reproducción máxima de *O. majusculus* en *L. maritima* es necesaria la presencia de presa. En general, los resultados demuestran que *L. maritima* proporciona recursos suficientes para la conservación de *O. majusculus* en periodos de escasez o ausencia de presa.
- *Lolularia maritima* mejora claramente la reproducción y supervivencia de *O. majusculus* en relación a las vainas de judía, especialmente en presencia de presa. Ello resalta los beneficios de usar una planta en flor que posee polen y néctar respecto a una estructura tan sencilla como son las vainas de judía.
- El cambio de planta hospedante no afecta a la reproducción de *O. majusculus* en presencia de presa. Ello implica que la inoculación de *O. majusculus* provenientes de crías comerciales sería susceptible de establecerse en *L. maritima*, siempre que hubiera disponibilidad de presa en el entorno.

Conclusiones al **Capítulo 2**:

- *Orius majusculus* y *O. laevigatus* depredan de manera efectiva trips en *L. maritima* en condiciones de laboratorio. Sin embargo, en invernadero, la diferente densidad de *O. majusculus* no afecta de manera distinta a las poblaciones de trips, disminuyendo estas de manera similar.
- *Orius majusculus* es capaz de reproducirse y establecerse con los trips como presa tanto en laboratorio como en invernadero. Sin embargo, los trips parecen una fuente de alimento de peor calidad que otras dietas altamente nutritivas como los huevos de *E. kuehniella*.
- *Lobularia maritima* permite el establecimiento de *Orius* spp., así como el control de trips por parte de dicho género depredador. Además *O. majusculus* se desplaza hacia el cultivo circundante tanto a altas como a bajas densidades del depredador. Así pues, *L. maritima* es una buena candidata para la conservación en campo de *O. majusculus.*

Conclusiones al **Capítulo 3**:

- Los efectos proporcionados por las distintas especies de plantas afectan de manera muy diversa al desarrollo de *O. insidiosus*, variando así su reproducción, supervivencia y estado nutricional. Estas diferencias se deben en parte al diferente acceso a los recursos florales y extra florales de las plantas.
- Un aumento en la diversidad vegetal permite optimizar la reproducción de *O. insidiosus* mediante el uso de distintos recursos vegetales, como son el alimento y los sustratos de oviposición. Algunas plantas representan un sustrato de oviposición muy adecuado para *O. insidiosus*, pero solamente cuando el depredador dispone cerca de fuentes alimenticias proteicas o lipídicas.
- *Orius insidiosus* responde de manera diferente tanto a las distintas especies vegetales como al acceso a los recursos florales y extra florales y por tanto es muy importante elegir la planta más apropiada para cada programa de conservación. Estos resultados demuestran que la combinación de distintas especies vegetales pueden tener efectos positivos en el desarrollo de *O. insidiosus*.

Conclusiones al **Capítulo 4**:

- Tanto *O. insidiosus* como *N. americoferus* detectan la presencia de la otra especie y modifican sus decisiones reproductivas en función de la ausencia o presencia de sus competidores.
- En presencia del otro depredador, *N. americoferus* aumenta el número de huevos puestos y cambia su preferencia por algunas estructuras dentro de la planta. En cambio, en presencia del otro depredador, *O. insidiosus* aumenta el número de huevos puestos sólo ligeramente y no cambia su preferencia por las estructuras donde realiza la puesta. Opuestamente, *O. insidiosus* modifica marginalmente el estrato dentro de la planta en el que pone sus huevos en presencia del otro depredador, mientras que *N. americoferus* no varía el estrato en el que pone sus huevos a pesar de la presencia de la otra especie depredadora.

Conclusiones al **Capítulo 5**:

- La identificación de ADN de tomate en el digestivo de insectos es posible con los marcadores moleculares diseñados, siendo estos marcadores específicos de tomate.
- Los marcadores moleculares diseñados permiten la detección de ADN de tomate inmediatamente después de la ingesta en todos los individuos analizados de todas las especies estudiadas, independientemente de su hábito alimenticio. Sin embargo, el tiempo de detección medio en el digestivo de los insectos varía en función del hábito alimentico, siendo mayor para los insectos masticadores.
- Los marcadores moleculares permiten la detección de ADN de tomate en *M. pygmaeus* recolectados en campo, obteniéndose porcentajes de detección más elevados en ninfas y hembras que en machos. En algunos individuos es posible además la detección tanto de planta como de presa, lo cual sugiere la existencia de una dieta mixta en este depredador omnívoro.

Conclusiones al **Capítulo 6**:

- La utilización adecuada de varios mantillos vegetales estabiliza notablemente los extremos ambientales en el suelo, mejora varios servicios al ecosistema en viña y puede mejorar la calidad de la uva y la cosecha.
- En general se encuentran más artrópodos (depredadores entre otros) y lombrices de tierra en los mantillos vegetales que en el suelo desnudo en ambos viñedos. Las poblaciones de *E. postvittana* y daños asociados durante la cosecha en uno de los viñedos son menores cuando se usan mantillos, observándose también un menor nivel de incidencia de botrytis. Sin embargo, no se observan variaciones en la depredación de *E. postvittana* en la parte aérea independientemente del uso de matillos en ninguno de los viñedos.
- Aunque los resultados varían dependiendo del viñedo y del tipo de mantillo usado, estos resultados sugieren que un uso apropiado de mantillos vegetales en viñedos confieren varios beneficios y merece ser estudiado en profundidad.

General introduction

Conservation Biological Control and Habitat Management

Agriculture during the 20th century has focused on very intensive productions that depend on external inputs such as fertilizers or pesticides. These practices have lead to a simplification of the landscape, being monoculture the better representative of the agriculture nowadays. Many authors have pointed out that crop diversity has suffered a decline that has resulted in a simplification of the landscape, and that the expansion of monocultures has diminished the abundance of natural enemies due to the suppression of food resources and shelter (Andow, 1991; Tscharntke *et al.*, 2007). Nevertheless, in the last years there has been an increasing interest in the application of more environmentally friendly techniques in agriculture (Gurr *et al.*, 2004).

Integrated Pest management (IPM) appears in modern agriculture as a need for substituting the excessive application of chemical products for other techniques less harmful to the environment. Specifically, IPM includes the use of biological, chemical or cultural methods as well as the use of biotechnology (Eilenberg *et al.*, 2001). One of these components is the biological control of pests, which consists of the use of natural enemies for maintaining pest populations to lower levels than would occur in their absence (Debach, 1974). According to Eilenberg *et al.* (2001), there are four kinds of BC: (1) Classical, or the intended introduction of an exotic biological control agent for pest control, (2) Inoculation, the intended introduction of a biological control agent for temporary pest control, but not permanently, (3) Inundation, the use of organisms to control pests with an exclusive control by the released organisms, and (4) Conservation, which implies the modification of the environment or existing practices to protect and enhance natural enemies to reduce the effects of pests.

Conservation Biological Control (CBC) involves the manipulation of the environment to enhance natural enemies' effectiveness, which can be achieved by means of reducing harmful conditions or improving favorable ones (Landis *et al.*, 2000). CBC is mainly based upon the exploitation of native natural enemies which are able to colonize crops when they are necessary (Barbosa, 1998; Pickett and Bugg, 1998), although this approach can be useful for conserving introduced natural enemies as well (Gurr and Wratten, 1999). In CBC the impact of natural enemies is maximized because pesticide mortality is minimized (Gurr *et al.*, 2000). Specifically, CBC consists of the creation of an appropriate environment for the natural enemies, taking into account the factors that influence them (Heinz *et al.*, 2004): environment (temperature, photoperiod or ventilation), pesticides, access to alternative food sources, plants effects (oviposition sites or shelters) and interactions with other natural enemies. During the last years, and due to its importance in enhancing the natural enemy's performance, more and more attention has been paid to this form of biological control (Jonsson *et al.*, 2010; Perdikis *et al.*, 2011).

Nowadays, agroecosystems are mainly formed by very unfavorable environments for natural enemies due to high levels of disturbance (Landis *et al.*, 2000); therefore, the creation of suitable environments for beneficial insects within the agricultural landscape is important. Habitat management, a part of the CBC approach, consists of selecting plants that provide natural enemies with resources such as non-host foods including pollen or nectar, alternative preys or hosts or shelter from adverse conditions (Landis *et al.*, 2000; Fiedler *et al.*, 2008). There are several forms of habitat manipulation approaches that may enhance natural enemies' numbers in crops which include beetle banks, flower sown strips, intercropping, ground cover management or mulches, among others (Landis *et al.*, 2000 and references therein; Thomson and Hoffmann, 2007). Of all those different strategies, the use of insectary plants to conserve beneficial insects has been greatly used (Hogg *et al.*, 2011; Nafziger and Fadamiro, 2011).

Natural enemies and plant resources in agroecosystems

Plants represent a universe for insects where they find not only food but also oviposition substrates, shelter or a place for mating. As a consequence, plants play a very important role for insects: they are considered a tool to sustain beneficial insects and, thereby, improvement in biological pest control is expected. In agroecosystems, an increase in biodiversity is related to an enhancement of natural enemies' performance and, therefore, to a reduction of pests (Andow, 1991). Nonetheless, it is important to identify and to provide the right diversity (Gurr *et al.*, 2005) because sometimes plant subsidies can benefit other arthropods apart from the desired beneficial insect (Araj *et al.*, 2009; Winkler *et al.*, 2009). This means that the most appropriate plant or plants species should be used among each different scenario.

There are many field studies that have shown that the presence of mainly flowering plants have implied an increase in the abundance of natural enemies (Alomar *et al.*, 2006; Fiedler and Landis, 2007a; Hogg *et al.*, 2011) or even and enhancement of pest control (Irvin and Hoddle, 2007; Alomar *et al.*, 2008; Pease and Zalom, 2010). In addition to the attractiveness that plants exert in natural enemies, it is well known that plant resources (such as pollen, floral or extrafloral nectar and plant sap) are used by natural enemies as food sources (Alomar and Wiedenmann, 1996; Wäckers, 2005). These plant subsidies can be used both as a complement to diet during times of prey abundance or even for survival on times when prey is scarce (Lundgren, 2009). There are many examples showing enhanced survival and reproduction of parasitoids and predators under controlled conditions as a result of provided plant resources (Taylor and Pfannenstiel, 2009; Lundgren *et al.*, 2010; Nafziger and Fadamiro, 2011).

However, besides the possible positive impact on beneficial insects, the addition of plants into agroecosystems could also have negative effects (Huang *et al.*, 2011). According to Gurr *et al.* (2005), there are several risks associated with the use of plants that should be evaluated before any introduction takes place: exotic plants that could become invasive, intraguild predation or competition associated with an increase in the number of natural enemies or the possible enhancement of species other than the desired beneficial insect. Sometimes herbivores can also profit from these plant subsidies by means of directly feeding on them (Baggen and Gurr, 1998; Hulshof *et al.*, 2003; Winkler *et al.*, 2009) or also caused by a reduction in prey consumption or parasitism (Skirvin *et al.*, 2007; Robinson *et al.*, 2008). In other cases, also other natural enemies (such as hyperparasitoids or intraguild predators) could benefit from these plant resources, which could elicit a reduction of the desired pest control (Araj *et al.*, 2009). Therefore, the addition of flowering plants into agroecosystems may inadvertently increase pest pressure when it is applied without caution. However, despite the fact that other insects, apart from the targeted natural enemy, can also profit from these resources is generally neglected and not many works have been carried out on this topic. Only selective food plants that promote the performance of natural enemies, but without favoring insects other than the targeted beneficial, should be used for being introduced in agroecosystems (Baggen and Gurr, 1998). It is important to know the extent to which an insectary plant that can harbor pest populations represents a risk for the crop: if natural enemies are able to control those pest populations on the plant, the risk for the crop could be disregarded. As a result, the study of the pest-natural enemy interactions on each possible companion plant should be studied in detail.

Arthropods lay their eggs in mainly plants and, as a result, oviposition decisions are frequently influenced by the plant characteristics. Some insects show clear preferences of oviposition between plants (Coll, 1996; Sanchez *et al.*, 2004; Lundgren and Fergen, 2006). In other occasions, within a plant species, distinct preferences for specific plant tissues have been shown too (Sigsgaard, 2004; Groenteman *et al.*, 2006; Lundgren *et al.*, 2008). Additionally, another factor that may influence the oviposition behavior of a predatory female is the presence of other natural enemies and the possible interactions that can take place, as it happens with the intraguild predation (Faraji *et al.*, 2001, 2002; Montserrat *et al.*, 2007). Even the possibility of an avoidance behavior between predators has been recently discussed (Lundgren, 2011). Plants are complex scenarios for insects where many different interactions can take place and the extent to which these interactions influence them should be studied in detail.

Plant feeding on the performance of omnivorous predators

In literature there are many studies that have documented the importance of plant-provided foods for parasitoids (Berndt and Wratten, 2005; Begum *et al.*, 2006; Lee and Heimpel, 2008) but the benefits of the plant provided subsidies for predators is a more recent subject of study. Notwithstanding, there has been a growing interest in the importance of plant foods in the performance of omnivores, which are predators that feed on both prey and plant resources (Coll and Guershon, 2002; Lundgren, 2009). Some omnivores are referred to as life-history omnivores because they consume plant and prey foods at different stages of their life (Coll and Guershon, 2002). Others use both plant and prey food throughout their lives and are defined as lifelong omnivores (Eubanks and Styrsky, 2005). Despite these differences, the biology and performance of both of them can be greatly influenced by the species or quality of the plant they feed on.

Plant feeding can influence the survival and development of immature predators (Cottrell and Yeargan, 1998; Eubanks and Denno, 1999), the adult longevity and fecundity (Limburg and Rosenheim, 2001; Beckman and Hurd, 2003), the survival of the predators in the absence of prey (Eubanks and Denno, 1999; Leon-Beck and Coll, 2007) or the suppression of prey populations, either positively (Gillespie and McGregor, 2000) or negatively (Robinson *et al.*, 2008). Of all the cited effects that plants exert on them, a very interesting characteristic of these omnivorous predators is that their plant feeding habits allow them to persist during times of prey scarcity (Eubanks and Denno, 1999; Leon-Beck and Coll, 2007). Under such a situation of prey absence, they are unlikely to starve or leave the habitat because they have other food sources available. However in a prey-free environment where there is no availability of plant food resources they may be more likely to leave the habitat due to the starvation situation. Therefore, the conservation (mainly achieved by means of plants) of these omnivores in or around the crops is desirable because they are able to survive at low prey densities with the consequence of slowing down the buildup of pests (Coll and Guerson, 2002).

There are several works published about different families of omnivorous predators feeding on plant sap (Stoner, 1972; Ruberson *et al.*, 1986; Lundgren *et al.*, 2008) or pollen (Corey *et al.*, 1998; Broufas and Koveos, 2000; Beckman and Hurd, 2003). However, research about the importance of nectar sources on predators has received little attention until recently and there is limited evidence to indicate that predators actually feed on nectar (Limburg and Rosenheim, 2001; Lundgren and Seagraves, 2011; Portillo *et al.*, 2012). Despite the fact that plants are known to be important food sources for omnivorous predators, the extent to which plant subsidies such as pollen, nectar or extrafloral nectar affect them are still poorly understood.

Molecular biology applied to the Conservation Biological Control

In agricultural landscapes dominated by annual crops, the establishment of natural enemies is more difficult than in other more permanent areas due to the ephemeral nature of these crops that are constantly interrupted (Alomar and Albajes, 2003). In those situations, arthropods suffer from a lack of food sources, shelter, mating and nesting sites. Therefore, the periodic destruction of crop and non-crop vegetation makes the establishment of predators and parasitoids that must re-colonize the fields time after time more difficult (Alomar and Albajes, 2003; Alomar *et al.*, 2006). As a result, the control of pest populations by predators during the critical early periods of crop growth may be diminished (Landis *et al.*, 2000). Hence, in these areas the conservation through habitat management of native natural enemies that are usually found in the area may facilitate the biological pest control (Alomar and Albajes, 2003; Alomar *et al.*, 2006). Therefore, the identification of proper insectary plants that are able to conserve and maintain natural enemies' populations near the crops is important.

The provision of appropriate insectary plants in or around crops may increase natural enemies' populations but they can also come from other sources from the surrounding area. These migrations boost biological control programs but do not allow either to correctly assess the benefits of the proposed insectary plants on natural enemies' performance or to identify other possible refuges (Corbett and Rosenheim, 1996). Moreover, it is not always possible to know the origin of the predators that colonize crops due to the fact that there are no identified refuges in the vicinity of the area (Alomar *et al.*, 2002; Gabarra *et al.*, 2004). Different marking and tracking techniques have been used to evaluate the movement and dispersion of arthropods between crop and non-crop habitats (Lavandero *et al.*, 2004; Scarratt *et al.*, 2008; Stephens *et al.*, 2008). However, those methods have a disadvantage for field studies due to the fact that either the plant or the insects must be marked beforehand. Other works implied the identification of arthropods' ingested meals by means of serological techniques, morphological identifications or chromatography (Heimpel *et al.*, 2004; Silberbauer *et al.*, 2004; Jones *et al.*, 2006). These methods present the inconvenience that they are laborious and time consuming for large field studies.

An alternative way to track movements of omnivorous predators from their refuges in the habitat to the surrounding crops would be the use of molecular methods. With this technique it would be possible through the identification of plant-ingested DNA as it is similarly done in predation gut content analysis studies. From the first studies published on predation analysis in the late nineties (Agusti *et al.*, 1999; Agustí *et al.*, 2000) there has been, throughout the last decade, a large amount of research carried out and it has become a very common topic. Nevertheless, the plant meal identification within insect guts is a rather new subject that has emerged during the last years (Miller *et al.*, 2006; Matheson *et al.*, 2007; Jurado-Rivera *et al.*, 2009). In those studies plant fragments were sequenced and compared for homologies in the BLAST database. Such procedures are a very useful method for the identification of plants ingested but they are not very practical for large field studies where a huge number of insects should be analyzed. Hence, the development of plant-specific primers to identify plant-ingested DNA within insects would be a much cheaper and suitable method, and therefore a seemingly promising technique. Additionally it could be used to detect feeding events such as predation or parasitism and plant feeding simultaneously, which would help to know the extent to which natural enemies mix plant and prey feeding in their daily diets.

Conservation Biological Control and Ecosystem Services

Conservation Biological Control practices can also be seen as part of the provision of Ecosystem Services (Fiedler *et al.*, 2008). The concept of ecosystem services (ES) was defined by Daily (1997) as the processes by which ecosystems "sustain and fulfill human life". Some works have demonstrated that conventional farming systems have decreased these ES compared with more sustainable systems, such as organic agriculture (Sandhu *et al.*, 2008). Nonetheless, there are some agronomic practices such as the use of mulches that may not only enhance the ecosystem services in crops but also the CBC by means of decreasing herbivore populations, increasing natural enemies or improving predation rates in the canopy.

Mulches have been shown to provide multiple ecosystem services such as weed control (Elmore *et al.*, 1997; Brown and Tworkoski, 2004), improved soil fertility (Agnew *et al.*, 2005) and soil water infiltration (Buckerfield and Webster, 2001), reduction in soil temperature fluctuations (Mundy and Agnew, 2002), enhancement of soil formation by earthworms

(Buckerfield and Webster, 1996) or even a reduction in the incidence of some diseases (Jacometti *et al.*, 2007a, b). In relation to CBC, the use of mulches can also provide pest management benefits, with reduction of herbivores or increased natural enemy populations (Mathews *et al.*, 2002; Brown and Tworkoski, 2004; Suckling *et al.*, 2006; Thomson and Hoffmann, 2007). All these benefits of mulching should be integrated and their value in increasing the biodiversity of soil invertebrates as well as improve the ecosystem functions should be evaluated together.

The study system

The application of CBC is especially useful in very intensive agricultural systems where the habitat is markedly unstable for natural enemies since crops are destroyed and initiated continuously and their establishment in the fields is usually difficult. As explained beforehand, a possible solution to this problem is the provision of alternative refuges for beneficial insects. On these changing environments, the use of omnivorous predators takes relevance due to the good capacity of survival of these natural enemies during times of prey scarcity (Eubanks and Denno, 1999).

A very important group of omnivorous predators are the members that belong to the order Hemiptera (Alomar and Wiedenmann, 1996). In this thesis, predatory Hemiptera belonging to the families Antochoridae and Miridae were selected due to their relevance in conservation biological control programs in important crops such as tomato or lettuce. *Orius majusculus* (Reuter) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) are Paleartic species commonly found in Europe (Riudavets, 1995) where they have been identified as native predators of the introduced and highly harmful thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Riudavets and Castane, 1998). Some authors have observed that the genus *Orius* spp. is an abundant spontaneous predator in lettuce where they can effectively control pests such as aphids or thrips (Arnó *et al.*, 2002); its maintenance around these fields is therefore important. Another important predator is *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae). It is found in several areas along the Mediterranean coast (Goula, 1986) where it preys on several pests. Moreover, it has been used to effectively reduce whiteflies populations and its use has been very successful in biological control programs in tomato (Avilla *et al.*, 2004). Another important Anthocorid is *Orius insidiosus* Say, a very important native predator in North America in crops such as corn, soybean or alfalfa of mainly thrips and aphids (Baez *et al.*, 2004; Rutledge and O'Neil, 2005; Seagraves and Yeargan, 2009). In some of these crops it shares the habitat with other predators such as *Nabis americoferus* Carayon (Hemiptera: Nabidae) (Coll and Ridgway, 1995; Clements and Yeargan, 1997), a generalist predator of Midwestern field crops. Due to the relevance of all these omnivorous predators as biocontrol agents in agroecosystems, they are used in conservation biological control programs and were selected for this thesis.

The first plant selected for the study and for several chapters of this Thesis is *Lobularia maritima* L, alyssum. One of the main reasons for its selection was that it has been observed that *Orius*. spp established and maintained well on this plant during times between crops (Alomar *et al.*, 2008). Moreover it is a Mediterranean plant with an extended flowering period (Pico and Retana, 2000) and it has been succesfully used in many conservation biological control programs.

Objectives

The overall aim of this project was to investigate the extent to which the use of vegetal resources may contribute to the conservation biological control through habitat management. To develop this general objective, several specific objectives were identified:

(1) To evaluate the use of *L. maritima* and the absence or presence of prey compared with a control (green bean pods) on the conservation of the omnivorous predator *O. majusculus*, as well as to study the effects of a host plant change on *O. majusculus* reproduction. This specific objective was addressed in the **Chapter 1**.

(2) To determine the control exerted by *Orius* spp. on *F. occidentalis* populations on alyssum as well as how this predation affects the reproduction of the predator. In addition, to evaluate the dispersion of *O. majusculus* from alyssum to a surrounding crop. This objective was addressed in the **Chapter 2**.

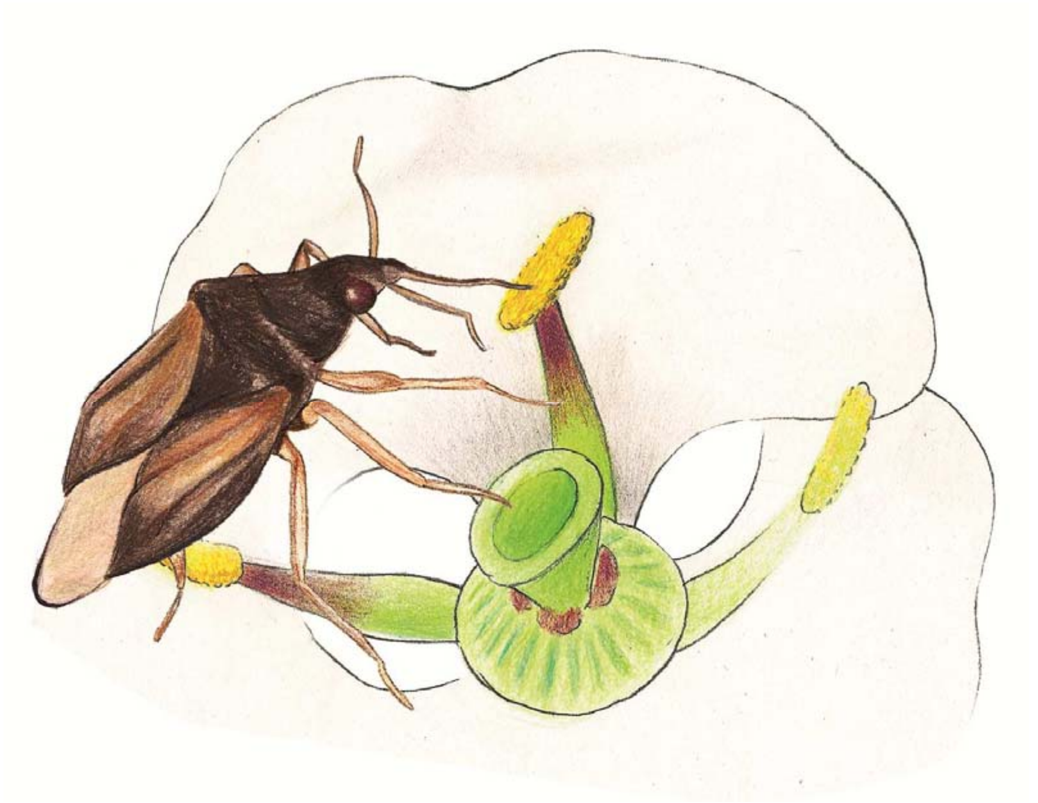
(3) To measure how plants that differ in their accessibility to the nectar and pollen sources affect the performance of *O. insidiosus* as well as to evaluate the extent to which this predatory bug is affected by an increase in plant diversity. This objective was addressed in **Chapter 3**.

(4) To evaluate the interactions among *O. insidiosus* and another predatory Hemipteran, *N. americoferus* and to study how these bugs alter their oviposition intensity and placement of eggs on a plant in the presence of competitors. This objective was addressed in **Chapter 4**.

(5) To develop tomato specific markers that allow the identification of tomato DNA within insects with different feeding types, as well as the digestion times of tomato within these insects. To evaluate the utility of these markers to detect tomato DNA within field collected insects. This objective was addressed in **Chapter 5**.

(6) To evaluate the potential of several mulches to increase the biodiversity of soil invertebrates as well as the enhancement of ecosystem functions in the soil in two contrasting vineyards. This objective was addressed in **Chapter 6**.

The role of omnivory in the conservation of predators: *Orius majusculus* (Hemiptera: Anthocoridae) on sweet alyssum



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This chapter has been submitted to Biological Control.

Front page: *Orius* spp. feeding on an alyssum flower

Resumen

En programas de control biológico por conservación, la provisión de los recursos necesarios (refugio, comida, lugares de puesta, etc.) es posible mediante el uso de plantas insectario. Esto es especialmente importante en el caso de los depredadores omnívoros que se alimentan tanto de presa como de planta y para los cuales la disponibilidad de recursos vegetales juega un papel muy importante en su conservación. Un depredador muy importante de trips y otras plagas en el mediterráneo es el chinche omnívoro *Orius majusculus* Reuter (Hemiptera: Anthocoridae). El objetivo de éste estudio fue el de evaluar como *Lobularia maritima* L. afecta la fertilidad, fecundidad y longevidad de *O. majusculus* en relación a distintos materiales vegetales (plantas de *L. maritima* y vainas de judía verde) y presa (huevos de *Ephestia kuehniella*). Además se estudiaron los efectos de la planta de origen de los individuos en la fertilidad de *O. majusculus.* La fecundidad en ambas plantas sin presa fue baja, sin embargo el hecho de añadir presa incrementó significativamente el número de huevos puestos, especialmente en el caso de *L. maritima.* La longevidad varió significativamente entre las distintas dietas, siendo la mayor la obtenida en la dieta de *L. maritima* con presa. La supervivencia en *L. maritima* sin presa no fue significativamente diferente de la obtenida en vainas de judía con presa, lo cual demuestra el potencial de *L. maritima* para conservar *O. majusculus* durante periodos de ausencia de presa. La fertilidad de las hembras de *O. majusculus* fue mayor en las dietas de ambos materiales vegetales cuando se incluyó presa en las mismas. Estos resultados demuestran que *L. maritima* puede proporcionar los recursos necesarios para el mantenimiento de *O. majusculus* en campo.

Abstract

In conservation biological control programs, provision of the required resources (shelter, food, oviposition sites, etc.) can be achieved by means of the use of insectary plants. This is especially important in the case of omnivorous predators that feed on prey and plant materials, and where the availability of plant resources plays a very significant role in their conservation. An important predator of thrips and other pests in the Mediterranean is the omnivorous bug *Orius majusculus* (Reuter) (Hemiptera: Anthocoridae). The objective of this study was to evaluate how alyssum, (*Lobularia maritima* L.) affects the fertility, fecundity and longevity of *O. majusculus* relative to different plant materials (alyssum and green bean pods) and prey (*Ephestia kuehniella* eggs). Additionally, the effects of the breeding colony of the individuals on the fertility of different strains of *O. majusculus* were studied. Fecundity on both plants without prey was low; however the addition of prey significantly increased the number of eggs laid, especially on alyssum. The longevity differed significantly among diets, being longer for alyssum with prey eggs. Survival on alyssum without prey was not statistically different from green bean with prey, which shows the potential of alyssum to conserve *O. majusculus* during times of prey scarcity. The fertility of *O. majusculus* females was significantly higher on the diets of both plant materials when prey was included. Our results showed that alyssum can provide necessary resource subsidies for the maintenance of *O. majusculus* in the field.

1. Introduction

Conservation biological control of pests, part of the ecological engineering concept (Gurr et al., 2004), involves manipulating the habitat of natural enemies to enhance their survival and fitness, thereby enhancing their effectiveness in crops (Barbosa, 1998). This is especially important in intensive agricultural systems such as those found in Mediterranean agriculture. The greenhouses or field crops of this area are characterized by annual production practices, and the discontinuity that comes along with these systems makes it difficult for natural enemy communities to persist in these environments (Gerling *et al.*, 2001; Alomar and Albajes, 2003). Notwithstanding, recent efforts to increase biodiversity and reduce habitat disturbance through the use of agronomic practices show promise in conserving natural enemy communities and the services they provide.

A way to conserve natural enemies within cropland is by means of insectary plants which provide them with the resources they need to survive (shelter, alternative food and oviposition sites). For example, the introduction of flowering plants in agricultural systems increases nectar and pollen resources that are required by some natural enemies (Lundgren, 2009). These flowering plants attract beneficial insects into cropland that could enhance the biological control of cropland pests. There are many studies that involve the use of plant resources by parasitoids either in the lab (Berndt and Wratten, 2005; Nafziger and Fadamiro, 2011) or in the field (Begum et al., 2006). There is a growing interest in the importance of these plant-provided foods in the life histories of true omnivores, which are predators that feed on both prey and plant resources (Coll and Guershon, 2002; Lundgren, 2009). The positive effects of pollen, nectar or the plant itself have been studied for some families of omnivorous predators (Limburg and Rosenheim, 2001; Beckman and Hurd, 2003; Pease and Zalom, 2010; Lundgren and Seagraves, 2011; Portillo *et al.*, 2012). Omnivorous predators can be particularly effective in controlling pests because they sustain themselves on plant-based foods when prey are scarce , although important questions remain unknown on the ecology of these interactions (Lundgren, 2009).

The genus *Orius* comprises a group of omnivorous predators that are used in Europe and around the world as biocontrol agents. The predatory bug *Orius majusculus* (Reuter) (Heteroptera: Anthocoridae) is a Palearctic species usually found in central and southern Europe (Riudavets, 1995), where it resides in several Mediterranean crops throughout the growing season (Goula et al., 1993; Riudavets and Castane, 1998) as well as in wild flora surrounding cropland (Bosco and Tavella, 2008). This polyphagous predator effectively preys on several pests (Alvarado et al., 1997; Montserrat et al., 2000; Tommasini et al., 2004) and it has been successfully used to control western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) in the field (Trottin-Caudal *et al.*, 1991; Fischer *et al.*, 1992). Having a broad diet is especially important in places where prey species fluctuate, as in ephemeral and disturbed agroecosystems of the Mediterranean area; *O. majusculus* appears to be a promising biocontrol agent in greenhouses of that area.

The perenial herb, *Lobularia maritima* (L.) Desv. (Brassicaceae), has several traits that make it a strong candidate as an insectary plant useful in conserving *O. majusculus* and it was therefore selected for this study. First, this plant has an extended flowering period of approximately 10 months (Pico and Retana, 2000); it is very attractive to natural enemies, and thus has potential as an insectary plant (Alomar et al., 2008; Hogg et al., 2011). Furthermore several parasitoids display greater fitness when allowed access to this plant (Berndt and Wratten, 2005; Begum *et al.*, 2006). Moreover densities of a predaceous bug were greater in tomato fields adjacent to an alyssum border (Pease and Zalom, 2010), and Bennison et al. (2010) advocate for the use of alyssum as a banker plant for *O. laevigatus*.

Biology studies of *O. majusculus* are only available on green bean pods and ivy or geranium leaves (Alauzet et al., 1992; Fischer et al., 1992; Tommasini et al., 2004). Green bean pods are also commonly used as an oviposition substrate for rearing of *Orius* spp. and they were therefore selected to be used as control. However, once adults are released in the field they face different environments and plants that may hamper their establishment. Prior experience can affect decisions by arthropods (Keasar et al., 2001), and a change of host plant can also be a critical factor in the establishment of phytophagous insects or influence natural enemy establishment (Lester *et al.*, 2000; Coyle *et al.*, 2011).

The objective of this study was to evaluate the relative contribution of *L. maritima* and the use of prey in the conservation of *O. majusculus*. Experiments were enacted (1) to determine the effects of plant and prey food resources on the fecundity and longevity of *O. majusculus* and (2) to test whether a change of host plant could affect *O. majusculus* reproduction.

2. Material and methods

2.1 Insects and plant material

Orius majusculus were reared in IRTA facilities. This colony was initiated with individuals collected from the fields in 2002 and is renewed every year with insects collected near the lab

(Barcelona, Spain). Two colonies were maintained on either alyssum plants (*L. maritima* Cv. Carpet of Snow) or green bean (*Phaseolus vulgaris* L. Cv. Helda) pods, both with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, under controlled conditions: 24.8±0.1ºC, 69±0.5 relative humidity (RH) and L16:D8 photoperiod. *Ephestia kuehniella* eggs were provided by Biotop (Valbonne, France).

Alyssum plants were obtained from a nursery and maintained at ambient conditions until they were used in the experiments. Green bean pods were obtained from a grocery store and kept in the fridge until used in the experiments or colony production.

2.2 Experimental procedures

2.2.1 Effects of plant and prey food resources on the fecundity and longevity of *O. majusculus*

To test the effects of different plant and prey food resources on the fecundity and longevity of *O. majusculus* five diets were used: (1) alyssum plants, (2) alyssum plants with *E. kuehniella* eggs, (3) green bean pods, (4) green bean pods with *E. kuehniella* eggs, and (5) water. Newly (≤48 h) emerged *O. majusculus* adults (one female and two males) were introduced into a 500 ml cup containing excess of one of the indicated diets; the cups were covered with a thrips-proof gauze (65 μm mesh size) held in place with a rubber band. Insects assigned to the alyssum and green bean treatments had been produced on plant material of the same type. The water-only treatment involved insects raised on green beans. For the green bean treatments we covered the ends of a 10 cm pod with paraffin. For the alyssum diets, a stem with 12-14 flowers was cut and wrapped with cotton and placed into a 10 ml water vial containing tap water and sealed with Parafilm. Care was taken to have prey-free plants and, prior to the experiments, those flowering stems were revised for thrips or any other pest. This structure was then placed through a hole made at the base of the 500 ml cup. For the water treatment, a 10 ml vial filled with tap water and a moistened cotton wick was used. In all diets containing prey, ca. 500-550 frozen eggs of *E. kuehniella* were stuck on the adhesive of a 1 cm^2 piece of Post-it ® and placed at the base of the cup. Three times a week the plant and insect material was replaced with fresh material and the numbers of missing, dead or alive insects were recorded. Dead or missing males were replaced with new ones. Replicates with missing females were discarded, and the experiment was repeated until 15 replicates per diet were obtained. The number of eggs laid on green bean or alyssum was counted under a microscope.

Longevity and fecundity were assessed until the female died. This experiment was carried out in the same controlled temperature chamber as the colony production.

2.2.2 Effects of prior experience on novel host plant acceptance

To test the effect of changing the host plant on the fertility of *O. majusculus*, females from both colonies (green bean and alyssum) were tested, both on their source and novel host. In a first phase of the experiment (phase-I) we exposed *O. majusculus* females (6-8 d old) from both colonies to the same plant (e.g. flowering alyssum or green bean pods) used in their rearing for 3 d (n = 15 per treatment). In a second phase (phase-II), the females were transferred for 3 d to one of three different diets: (1) alyssum plants, (2) alyssum plants with *E. kuehniella* eggs, and (3) green bean pods with *E. kuehniella* eggs (n=5 per treatment). This resulted in six treatments (Table 1.1). Similar to the first experiment, flowering alyssum were examined for thrips and alternative prey. The number of *O. majusculus* nymphs was counted 10 d after the removal of the *Orius* females in each of the different diets. The experimental unit for the alyssum diets consisted of a rooted flowering stem potted in a plastic tube (10 cm high and 3 cm diameter) and inserted through a hole in the base of a 1 l plastic cup. The green bean pod was placed on the base of the previously described plastic cup. In all treatments plastic cups were covered with thrips-proof gauze. In all diets containing prey, *E. kuehniella* eggs were added as previously described.

Table 1.1: Design of the experiment to test the effects of prior experience on novel host acceptance.

Treatment	*n*	Phase-I	Phase-II
AE-AE	5	Alyssum+prey	Alyssum+prey
AE-A	5	Alyssum+prey	Alyssum
AE-GBE	5	Alyssum+prey	Green bean+prey
GBE-AE	5	Green bean+prey	Alyssum+prey
GBE-A	5	Green bean+prey	Alyssum
GBE-GBE	5	Green bean+prey	Green bean+prey

2.3 Statistical analysis

A one-way ANOVA was used to determine differences in mean longevity and a two way ANOVA (with plant and prey presence as main factors) was used to determine the mean fecundity as well as the duration of the oviposition period per female among treatments; means were separated with the Tukey test (P<0.05). Longevity and fecundity data were log and square-root transformed, respectively, to ensure the homoscedasticity of variances. Survival analysis was used to compare the effect of the different diets on predator longevity: the Kaplan-Meier survival curves were plotted and the long rank test was carried out to test for overall differences between treatments. All the analysis were carried out with SAS Enterprise Guide 4.2.

To determine whether the fertility of *O. majusculus* is affected by the host plant, we compared the number of nymphs among both phase-I hosts by a one-way ANOVA. To determine the effect of changing host and of the absence of prey, we used a two-way ANOVA with phase-I and phase-II treatment as main factors (Table 1.1). Data were square-root transformed to ensure the homoscedasticity of variances. Means were separated with the Tukey test.

3. Results

3.1 Effects of plant and prey food resources on the fecundity and longevity of *O. majusculus*

Mean longevity of females was significantly affected by the host and prey treatments (df=4, F=50.76, P<0.0001). *Orius majusculus* survived longest on alyssum with prey, followed by alyssum without prey and green bean with prey (Table 1.2). Females on water or prey-free green beans only survived 1 wk. The survival rate also varied among treatments ($\chi^2 = 83.86$, df = 4, P < 0.0001) (Figure 1.1).

Table 1.2: Longevity of *O. majusculus* adult females fed on different diets (mean±s.e.). Means followed by the same letter do not differ significantly (α=0.05)

Diet	Longevity (d)		
	Mean±SE	Maximum	Minimum
Alyssum+prey	40.60±2.45 a	58	25
Alyssum	25.60 ±3.28 b	49	7
Green bean+prey	17.40±1.72 b	28	4
Green bean	7.60±0.48 c	9	2
Water	7.13±0.40 c	9	4

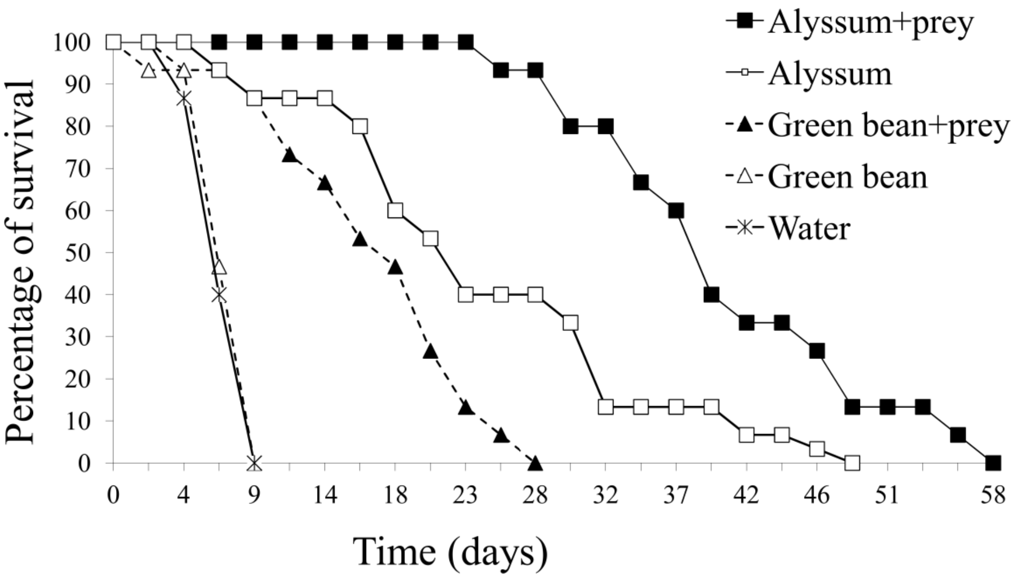

Figure 1.1: Survival rate of *O. majusculus* females fed on different diets over time (n=15).

Mean total fecundity and the duration of the oviposition period were significantly affected by the oviposition plant (*df*=1, *F*=21.40, *P*<0.0001; *df*=1, *F*=59.99, *P*<0.0001) and the presence of prey (*df*=1, *F*=387.11, *P*<0.0001; *df*=1, *F*=64.25, *P*<0.0001), but there was no interaction between these factors (*df*=1, *F*=3.90, *P*=0.0531; *df*=1, *F*=2.63, *P*=0.1106). The highest fecundity of *O. majusculus* was obtained on alyssum with prey, followed by green bean with prey. Fecundity on both plants without prey were similarly low (Table 1.3). Females confined on alyssum with prey had the longest oviposition period (56 d; Figure 1.2); those fed prey-free alyssum laid eggs for 46 d and those fed prey on green bean had the much shorter oviposition period of 25 d; finally those on prey-free green bean only laid eggs during 7 d.

Table 1.3: Fecundity of *O. majusculus* adult females fed on different diets (mean±s.e.). Means followed by the same letter do not differ significantly (α=0.05)

Diet	Total eggs/female		
	Mean±SE	Maximum	Minimum
Alyssum+prey	274.87±22.89 a	399	137
Alyssum	12.47±3.74 c	51	0
Green bean+prey	158.27±16.40 b	240	31
Green bean	4.07±1.45 c	16	0

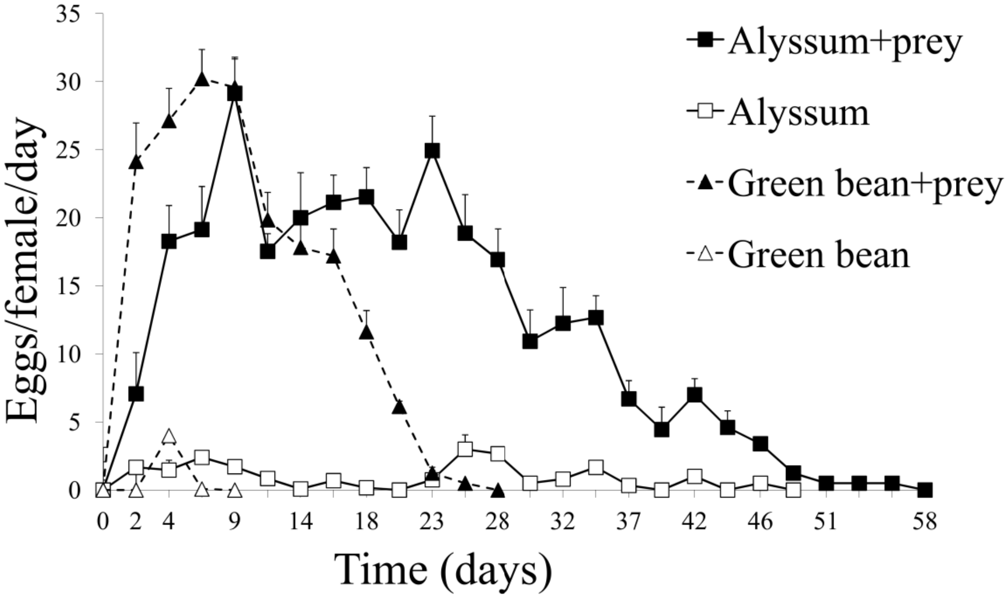

Figure 1.2: Mean (SEM) daily oviposition rate of *O. majusculus* fed on different diets (n=15).

3.2 Effects of prior experience on novel host plant acceptance

During phase-I (where *O. majusculus* females from both colonies were exposed to the same plant they bred), *O. majusculus* laid significantly more eggs on green bean than on alyssum plants (*df*=1, *F*=7.28, *P*=0.0117). The mean number of nymphs per day, per female in this phase-I was 2.79±0.48 and 4.29±0.41 when alyssum and green bean were the original plants, respectively. However, the number of eggs in phase-II was no longer affected by the colony of origin (*df*=1, *F*=0.36, *P*=0.5542), but only by the different diets they were exposed to during the phase-II (*df*=2, *F*=33.87, *P*<0.001). The interaction of both factors was not significant (*df*=2, *F*=0.19, *P*=0.8262). Females tested on green bean with prey or on alyssum with prey produced similar number of nymphs independently of their origins (Figure 1.3), and fertility of individuals from both colonies was significantly reduced when tested on alyssum plants without prey.

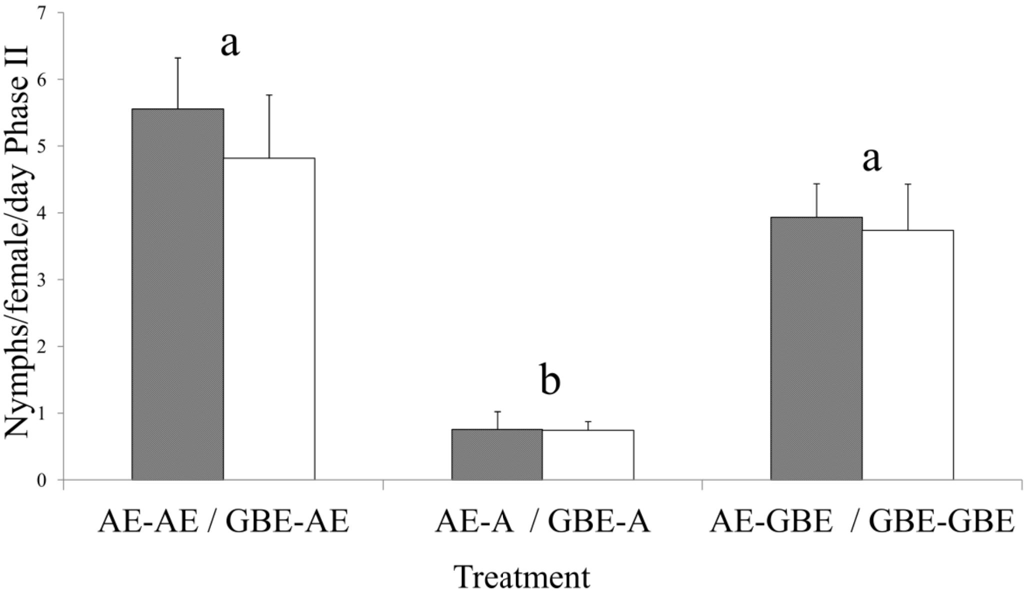

Figure 1.3: Fertility of *O. majusculus* females as affected by rearing colony and subsequent transfer to the same or a novel plant (mean+se). Coloured and white bars are treatments whose plant origin was alyssum and green bean, respectively. Uppercase letters show differences between treatments with green bean as their rearing plant, lowercase letters show differences between treatments with alyssum as their rearing plant. Means followed by the same letter do not differ significantly (Tukey's test; α=0.05).

4. Discussion

Alyssum without prey supports some reproduction by *O. majusculus*, but maximum reproduction on this plant by this bug is only possible in the presence of prey.

When *O. majusculus* individuals were provided with only water or green bean pods they lived for approximately 1 wk and produced few eggs. A similar result with green bean pods was shown for *O. insidiosus* (Richards and Schmidt, 1996a). However, survival in the prey-free treatments was dependent on plant quality. When alyssum stems and flowers were provided as a food source, mean female longevity and lifetime fecundity were at least three times greater than for bugs raised on green bean pods or the water only. Additionally, individuals on alyssum kept laying eggs constantly throughout their lives, which implied a much longer period of oviposition. Predators may profit from a combination of plant components (plant sap, nectar, pollen or seeds) to obtain essential nutrients and derive more than just water from plant feeding (Coll and

Guershon, 2002). In general, the persistence of *Orius* spp. in several crops at low prey densities has been attributed to the existence of pollen sources, and lack of pollen may explain limited success in their establishment (Vacante et al., 1997). *Orius* spp. nymphs are known to benefit from feeding on several plant resources, including pollen (Naranjo and Gibson, 1996; Coll, 1998; Lundgren, 2009). However the benefits of plant-only diets for *Orius* spp. adults seem to be limited and adults provided with diets of only plant parts (seedlings, leaves or pods) or together with pollen usually show reduced survival and fitness than when supplementing the plant diet with prey (Chyzik *et al.*, 1995; Funao and Yoshiyasu, 1995; Cocuzza *et al.*, 1997).

In our case, the addition of prey in alyssum and green bean pods significantly increased the mean longevity of the females, with the longest mean longevity obtained on alyssum being more than twice than those raised on green bean. Adding prey to the plants strongly increased the mean lifetime fecundity too, particularly for alyssum, where 1.75 times more eggs were laid than on green bean. High longevities and fecundities have also been recorded for *O. majusculus* with *E. kuehniella* eggs on other plant structures such as geranium and ivy leaves (Alauzet et al., 1992; Fischer et al., 1992), but also on green-bean pods (Tommasini et al., 2004). The effect of the addition of prey was especially marked on green bean, where the females' longevity on the green bean without prey diet was not statistically different from water. This effect however was not so stark on alyssum. This high quality plant already provides nectar and pollen, thereby supporting longevity and washing out some of the benefits of adding prey to this plant species. Although prey is important, it is also important to stress that the mean longevity of *O. majusculus* females on prey-free alyssum was longer than on green bean pods subsidized with prey; even if there were no statistical differences among them. This suggests again that alyssum seems to provide more resources than a mixed diet of green bean pods and prey that has traditionally been considered of good quality. Alyssum with prey not only ensured longer survival and longevity but also extended the oviposition period. Although oviposition on both diets peaked at similar times, the oviposition rate on alyssum continued over a much longer time than on green bean pods with prey. Such an extended oviposition period indicates the potential of alyssum plants both, with or without prey, as a tool for sustaing the populations of *O. majusculus* during periods of prey scarcity, and help prime the reproduction system to better exploit prey when it becomes available.

The results suggest the relative importance of plant-based factors over prey in some life-history traits of an omnivorous predator. Plant characteristics still seem to determine survival and fecundity of *Orius* spp bugs, even when plants are subsidized with prey. Potentially, the benefits of plant-feeding should be relatively minor when insect eggs are added as prey (Naranjo and Gibson, 1996), but the results show that this is not so: *O. majusculus* can obtain more resources from alyssum than on green bean pods when prey is present. Plant characteristics are known to play an important role in oviposition by *Orius* spp. (Coll, 1996; Seagraves and Lundgren, 2010) and, as habitat generalists, plant-based cues seem to over-ride prey-based cues (Lundgren, 2011). Overall, the stronger benefits of alyssum probably reflects the benefits of using entire plants versus green bean pods for producing and conserving natural enemies. The positive effects of using flowers over green bean pods and other plant structures has been shown for the congener *O. insidiosus*. Mendes et al. (2005) and Carvalho et al (2010) showed that fecundity and longevity of *O. insidiosus* were higher on a diet of flowers (*Bidens pilosa* L.) compared to other non-flowering plant species or plant parts, all with Lepidoptera eggs as prey. The higher oviposition on inflorescences was attributed to the higher abundance of prime oviposition sites (growing stems, protected sites or flower petioles) in *B. pilosa*. But it could also be due to morphological characteristics of the plant (Lundgren and Fergen, 2006).

The results of the host-plant change experiment show that females successfully oviposited on both plants when subsidized with prey, independently of their prior experience in the colony source. In phase-I of the experiment, the number of nymphs was significantly higher on green-bean pods with prey than on alyssum with prey. The fact that initially there were significant differences in fertility among both plants can be related to the different initial oviposition rates of *O. majusculus* observed on both host plants. Females as used in this experiment (6 to 8 d old), show a higher initial oviposition rate on green bean than on alyssum. In phase-II however, the number of nymphs obtained was no longer affected by the source colony but only by the phase-II treatments, indicating that prior exposure to another plant does not seem to influence oviposition by *O. majusculus*, and females accept host-plants that they had not experienced. This is important when releasing this predator in the field as it would assure a good survival of *O. majusculus* on alyssum when they come from commercial releases. The number of nymphs produced on green bean pods and alyssum with prey were not different, and fertility was only significantly reduced when females had been transferred to alyssum plants without prey. This latter result reinforces the importance of prey to ensure a full reproductive output in *O. majusculus,* although egg laying continues even in absence of prey. In regards to the impact of prey, it is worth mentioning that in both experiments prey was distributed over the bottom of the experimental cages so that predation and oviposition were physically separated. Even with this physical segregation the effect of prey on the reproduction of *O. majusculus* was remarkable.

5. Conclusions

Allysum improves the survival and fitness of *O. majusculus* in relation to green bean pods, and these benefits are even greater when prey is added to the system. The benefits of a mixed diet in the longevity and reproduction of *O. majusculus* follow those patterns observed for other omnivores (Eubanks and Denno, 1999; Coll and Guershon, 2002; Lundgren, 2009). Insects from colonies reared on green bean pods can be used to establish a population in a companion plant such as alyssum, where *O. majusculus* can sustain itself by plant-feeding, and could therefore be established in fields on companion plants even before crops are in the field. Adding factitious prey would ensure early population development until other prey establishes in the plant. However, it is also important to determine to what extent companion plants may not promote crop pests (Winkler et al., 2010), and what is the control such a polyphagous predator may exert on them (Pumariño and Alomar, submitted).

Acknowledgements

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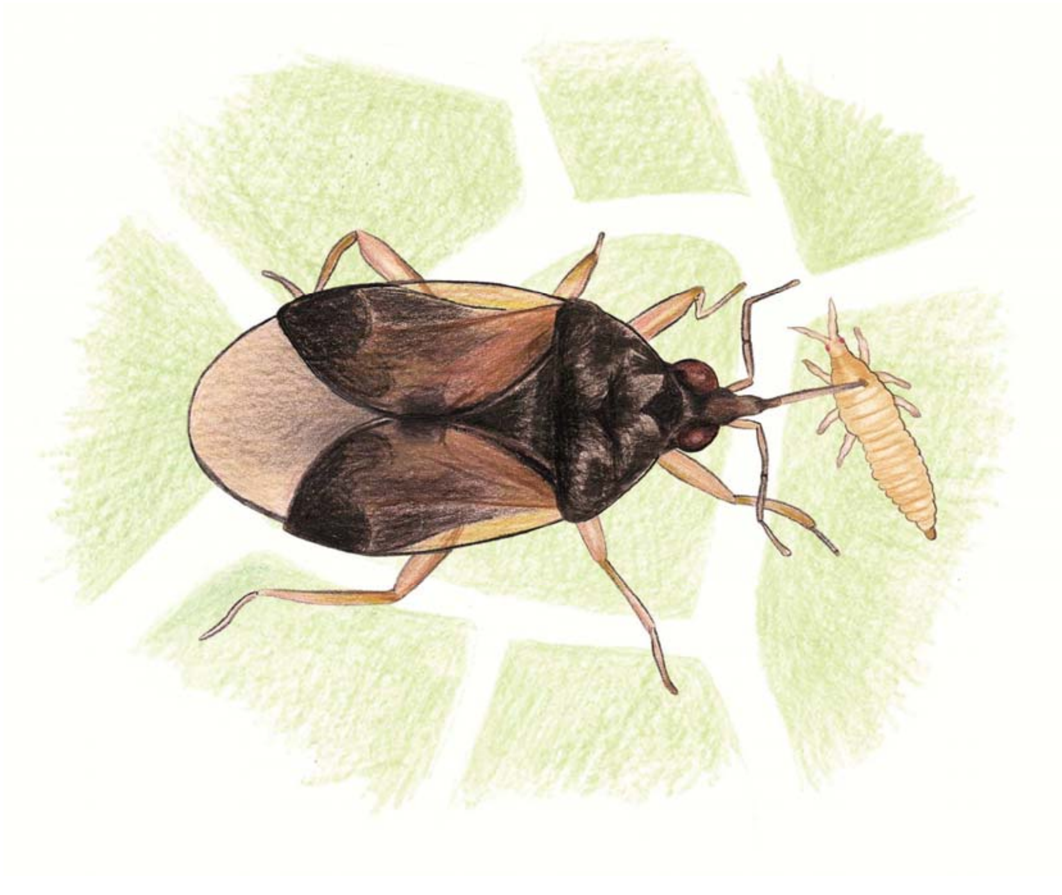
Assesing the use of *Lobularia maritima* as an insectary plant for the conservation of *Orius* spp.: biological control of *Frankliniella occidentalis*



Lorena Pumariño and Oscar Alomar

This chapter has been submitted to BioControl

Front page: *Orius* spp. feeding on a thrips nymph

Resumen

El uso de plantas con flor se emplea para la conservación de enemigos naturales en campo. Sin embargo dichas plantas pueden a su vez albergar poblaciones plaga y poco se sabe acerca del control de dichas plagas en las plantas insectario como para que no representen un riesgo para los cultivos. En este capítulo se ha estudiado el control del trips *Frankliniella occidentalis* (Pergande) ejercido por diferentes especies del género Orius en *L. maritima*. Los resultados de este trabajo demuestran que *Orius majusculus* (Reuter) y *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) depredan activamente al trips *F. occidentalis* en condiciones de laboratorio. Además *O. majusculus* es capaz de reproducirse cuando se alimenta de dicha presa en condiciones de laboratorio. En condiciones de semi-campo se encontraron números de trips menores en los tratamientos con altas densidades del depredador, aunque no significativamente distintos de los encontrados en los tratamientos con bajas densidades del depredador. Los resultados de este estudio indican que *L. maritima* permite el establecimiento de *Orius* spp. y el control de trips y que por tanto podría ser seleccionada para ser usada como planta insectario para la conservación de *Orius* spp.

Abstract

Flowering plants are used to conserve natural enemies in the field. However these plants may also sustain pest populations and little is known on the control of those pests so that they do not represent a major risk to the crop. Here, we study the control of *Frankliniella occidentalis* (Pergande) exerted by *Orius* spp. on alyssum. The results of this work show that *Orius majusculus* (Reuter) and *Orius laevigatus* (Fieber) actively prey on *F. occidentalis* under microcosm conditions. *Orius majusculus* is also able to reproduce when feeding on this prey in these same conditions. In semi-field conditions, lower numbers of thrips were found in the cages with high Orius densities although not statistically different from the low Orius density treatment. The results obtained in this study indicate that alyssum allows the establishment of *Orius* spp. and the control of thrips and could be selected as an insectary plant for *Orius* spp. conservation.

1. Introduction

Many predators and parasitoids benefit from feeding on plant-provided resources such as pollen, nectar, seeds or plant sap (Alomar and Wiedenmann, 1996; Wäckers, 2005). These vegetal resources can be used both as a complement to diet when prey is abundant or even for survival during times of prey scarcity (Lundgren, 2009). As a consequence, the use of flowering plants to enhance the fitness and success of natural enemies in crops has received more attention in recent years. Many plant species have been successfully used to improve the abundance and performance of beneficial insects either in the lab or in the field (Pease and Zalom, 2010; Lundgren and Seagraves, 2011; Nafziger and Fadamiro, 2011).

However, adding plants to an agroecosystem may also result in negative consequences that should be considered before the use of plant resources is applied (Huang *et al.*, 2011). Gurr *et al.* (2005) proposed some associated risks that should be evaluated: the use of exotic plant species that can become invasive, the intraguild competition and predation associated with an increase of natural enemies, as well as the risks of enhancement of species other than the desired beneficial (e.g., pest, other predators, parasitoids or hyperparasitoids). Little attention has been paid yet to the benefits to insects of adding floral resources other than the targeted beneficial, even though pests can benefit from those plant subsidies, too (Baggen and Gurr, 1998; Robinson *et al.*, 2008; Winkler *et al.*, 2010). Candidate insectary plants should improve the natural enemy fitness but without favouring pests or unwanted insects. Therefore, the most appropriate plant species for each specific case of study should be selected.

Species of the omnivorous genus *Orius* spp. are used and commercialized worldwide as biocontrol agents. They are important predators of thrips (Blaeser *et al.*, 2004), aphids (Rutledge and O'Neil, 2005), mites (Husseini *et al.*, 1993), whiteflies (Arnó *et al.*, 2008) and Lepidoptera eggs or larvae (Zaki, 1989). Two of the Paleartic species found in Europe are *Orius majusculus* (Reuter) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) (Riudavets, 1995). They have been identified as native predators of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Riudavets and Castane, 1998), which is an economically important pest in many crops and ornamentals, mainly in greenhouses. Surveys on weeds that may serve as sources of thrips for crops indicate the presence of several generalist predators that may be able to control thrips populations in some of these weeds and thus enhancing functional biodiversity (Ripa *et al.*, 2001; Silveira *et al.*, 2005; Atakan, 2010; Atakan and Tunc, 2010). However, the interactions between thrips and generalist predators on non-crop plants have not been studied. As such, the addition of flowers (i.e. pollen and nectar) in an agroecosystem could affect both the predator and the prey. Hulshof *et al.* (2003) observed that pollen from some plant species can be a food source for *F. occidentalis*, increasing its fecundity and growth rate. The addition of pollen diminished the predation ability of *O. laevigatus* on thrips (Skirvin *et al.*, 2007). Therefore, as flowering plants can serve as a food source for both the predator and the pest, the impact of these plants on arthropods populations should be taken into account in biological control programs.

Alyssum, *Lobularia maritima* (L.) Desv. (Brassicaceae), is a Mediterranean plant that has been proposed as an insectary plant to conserve natural enemies (Fiedler and Landis, 2007a; Alomar *et al.*, 2008; Hogg *et al.*, 2011). Previous work (chapter 1) has shown that *O. majusculus* can survive and also reproduce on alyssum and thus could be a good candidate for the conservation of this predator in the field in times of prey scarcity. However, this plant also sustains *F. occidentalis* populations in field conditions (personal observation) and could represent a source of thrips infesting nearby crops. Bennison *et al.* (2010) argued that alyssum can be used both as a trap plant for *F. occidentalis* as well as a banker plant to support *O. laevigatus* populations. Therefore, the extent to which alyssum is able to sustain *Orius* spp. populations without enhancing *F. occidentalis* populations should be studied in more detail.

The main objective of this study was to evaluate the control of *F. occidentalis* populations by *Orius* spp. on alyssum, and how this predation affects the reproduction of the predator. For this, we conducted three different experiments under microcosms and semi-field conditions. First we determined the predation of *O. majusculus* and *O. laevigatus* at two different densities of *F. occidentalis* on flowering alyssum stems and compared it with predation rates on green bean pods. Then we determined the capacity of *O. majusculus* to suppress thrips population growth on alyssum plants at two different thrips densities as well as the reproduction ability of the predator in the system. Finally, we evaluated the capacity of *O. majusculus* to control thrips populations and establish on alyssum plants and lettuce in greenhouse cages as well as the predator dispersion to the crop.

2. Material and methods

2.1 Sources of insects and plants

Orius majusculus, *O. laevigatus* and *F. occidentalis* were reared in IRTA facilities. These colonies were initiated with field individuals and they are renewed every year with insects collected near the lab. Both colonies were maintained on green bean (*Phaseolus vulgaris* L.) pods under controlled conditions: 25 ± 2 ºC, 70 ± 10 % relative humidity (RH) and a L16:D8 photoperiod. The predator colony was supplied with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs while the thrips colony was supplied with commercial pollen. *Ephestia kuehniella* eggs were provided by Biotop (Valbonne, France).

Alyssum plants were obtained from a nursery and maintained at ambient conditions until they were used in the experiments. Green bean pods were obtained from a grocery store and kept refrigerated until used in the experiments or in the rearings.

2.2 Predation of *F. occidentalis* by *O. majusculus* and *O. laevigatus* on alyssum

To evaluate the control of *F. occidentalis* by both predators, two thrips densities were tested (5 or 30 nymphs), both with or without Orius, on two plants (alyssum or green bean pods). This was repeated for both Orius species, *O. majusculus* and *O. laevigatus*. Newly emerged (≤ 48 h old) predator females were left for 24 hours either in a cylinder with an alyssum plant or in a glass pot with green bean pods, both with *ad libitum F. occidentalis* second instar nymphs. Afterwards they were starved in a glass pot with only water for 24 h before being used in the experiment. The starved females that had previously been on alyssum plants or green bean pods were used in the alyssum or green bean pods treatments, respectively.The experimental units consisted of a plastic cup (118 ml) with its plastic lid, which had a 1 cm^2 hole covered with surgical tape. Plant material was placed inside each unit. For the green bean pod treatments, 6 cm long pieces of pods with the extremes covered with paraffin were used. For the alyssum treatments, stems with 8-10 flowers were cut and wrapped with cotton and placed into a 10 ml water vial containing tap water and sealed with Parafilm. This structure was then fit in a hole made at the base of the cup. Five or 30 second instar *F. occidentalis* were placed in each arena. Two hours after the introduction of the thrips, one starved *O. majusculus* or *O. laevigatus* female was introduced in the with-predator treatments. Twenty-four hours later the cups were opened to determine the number of surviving thrips. Twenty replicates of each predator species, prey density and plant were conducted (total of 320 observations). The experimental conditions were the same as in the rearings.

2.3 Control of thrips population growth on alyssum plants by *O. majusculus*

To evaluate the control capacity of *O. majusculus* on a growing thrips population under microcosm conditions, 20 or 80 adult *F. occidentalis* were confined during 8 days on a flowering alyssum stem with or without a single *O. majusculus* female. To evaluate the relative benefits of thrips predation on the reproduction of *O. majusculus*, we added a treatment of alyssum without prey, and another of alyssum with *E. kuehniella* eggs, a highly nutritious prey. Finally, we also added a control treatment of alyssum but without adding neither thrips nor Orius. All treatments were replicated 8 times. The experimental unit consisted of a potted alyssum plant (10 cm diameter) enclosed within a plastic cylinder (2.18 l). Every plant was watered prior to the experiments and the pots were wrapped with plastic film to maintain humidity and to avoid insects moving into the soil. Care was taken to provide similar surface areas and numbers of flowers in each replicate. Adult *F. occidentalis* (5-10 d old) and 5-7 d old *O. majusculus* females starved for 24 h were used in the experiments. Twenty or 80 thrips were introduced on each plant in the respective treatments with thrips. Twenty-four hours later, one *O. majusculus* female was introduced in the treatments with predator. In the treatment with *E. kuehniella*, we added eggs *ad libitum* affixed to a Post-it® (2 pieces of ≈1cm² distributed in the middle part of the alyssum plants). Four days later all replicates were carefully opened, the plants were watered and fresh *E. kuehniella* was added to the corresponding treatment. Eight days after the introduction of *O. majusculus*, the introduced predators were removed and the number of *O. majusculus* nymphs and thrips (adults and nymphs) was counted and removed from each replicate. The plants were watered, fresh *E. kuehniella* was added in each replicate, and the units were closed again. Six days later, the experimental units were opened to count any newly emerged Orius nymphs, and thrips adults and nymphs. The results obtained in these two countings were pooled and the total number was analyzed.

2.4 Suppression of thrips population growth by *O. majusculus* in semi-field conditions

To evaluate the suppression of a thrips population on alyssum and a lettuce crop in semi-field conditions, two experiments were carried out in an experimental greenhouse at IRTA. The greenhouse had 8 cages (2.50 x 5.20 x 2.30 m) covered with a cloth mesh. In each cage, 4 alyssum plants were placed in the center, surrounded by 30 lettuces at a distance of 1.75 to 2.40 m. Two different treatments were applied: (1) only thrips and (2) thrips together with *O. majusculus*. Both treatments were replicated 4 times with a fully randomized design.

The first experiment was carried out during March and May, 2011 (62.3 ± 0.2 % mean relative humidity, and 29.3 ± 0.6 ºC and 13.2 ± 0.3 ºC mean maximum and minimum daily temperatures, respectively). Two hundred first or second instar thrips larvae were introduced on the alyssum plants of all cages. Forty-eight hours later, 5-7 d old *O. majusculus* (8 males and 12 females) were introduced on the alyssum plants of the respective cages. To determine whether there was an initial non-intended infestation, two alyssum inflorescences were collected from each cage before any insect was released. After the predators were introduced, the alyssum plants were sampled weekly during eight weeks. Four inflorescences were collected on each cage during the first four weeks, and eight inflorescences for the remaining weeks. The number of thrips and *O. majusculus*, both adults and nymphs, were counted. The lettuces were sampled three times at weeks 4, 7 and 8. In every one of those samplings, four lettuces were collected per cage, all the thrips and *O. majusculus* nymphs and adults were counted in the laboratory, and the results of these samplings were pooled. At the end of the experiment (week 8), additional intensive samples were taken on both, alyssum and lettuce. Alyssum plants were sampled at two consecutive days. Each day, the plants were shaken three times over a white tray and all fallen *O. majusculus* adults and nymphs were collected. This was repeated in four sampling points per cage, and all eight samples per cage were pooled. Ten lettuce plants were cut and opened *in situ* over a white tray and all the *O. majusculus* nymphs and adults were counted.

The second experiment was carried out during May and June, 2011 (59.4 ± 0.2 % mean relative humidity, and 32.3 ± 0.4 ºC and 13.9 ± 0.4 ºC mean maximum and minimum daily temperature, respectively). The procedure was analogous to the one described above, except that this time 16 males and 24 females of *O. majusculus* were introduced into each cage. As in the first experiment, an initial sampling and posterior weekly samplings (6 times) were carried out. In each of those samplings, 8 alyssum inflorescences per cage were collected. Again, an additional more intensive Orius sample was taken on the alyssum plants. The lettuce plants were only sampled at the end of the experiment with the two methods previously described.

During the monitoring in both greenhouse trials, *O. majusculus* was only found in three cages in the first experiment, but in all cages of the second experiment. We therefore calculated the number of cumulative insect-days (Ruppel, 1983) of *Orius* on alyssum for each cage and experiment. The treatments were then reorganized into two *O. majusculus* densities: ‘high Orius’

(23.63 ± 7.13 and 17.72 ± 0.97 Orius-days for the first and second experiment, respectively), and 'low Orius' (0 and 1.63±0.27 Orius-days for the first and second experiment, respectively).

2.5 Statistical analysis

The predation of *O. majusculus* and *O. laevigatus* on *F. occidentalis* nymphs on alyssum stems and green bean pods during a 24 h period was determined with the GLM procedure, with plant species, thrips density and Orius presence as factors. Data were checked for normality and homoscedasticity, and both *O.majusculus* and *O. laevigatus* numbers were root transformed before analyses. To determine differences among treatments, data sets with significant *F*-values were analyzed using the Tukey test ($P<0.05$).

One-way ANOVA was used to determine the control of thrips population growth on alyssum plants with *O. majusculus* as well as the reproduction of *O. majusculus*. To ensure homoscedasticity, the numbers of *O. majusculus* nymphs were root transformed and the numbers of thrips adults and nymphs were log+1 transformed. The differences among treatments were determined with the Duncan test ($P<0.05$).

The control of thrips population growth with *O. majusculus* on alyssum plants in semi-field conditions was evaluated with repeated measures ANOVA. The numbers of thrips and *O. majusculus* found on lettuce as well as the predators found on the more intensive samplings in lettuce and alyssum were compared among treatments with Student's t-test. Data were checked for normality and homoscedasticity and transformation of the data was not necessary. To determine differences among treatments, data sets with significant *F*-values were analyzed using the Tukey test ($P<0.05$). All the analyses were carried out with SAS Enterprise Guide 4.2.

3. Results

3.1 Predation of *F. occidentalis* by *O. majusculus* and *O. laevigatus* on alyssum

Both *O. majusculus* and *O. laevigatus* significantly reduced the number of surviving thrips either in alyssum plants or in green bean pods and at both thrips densities. In both cases there was a significant thrips density * Orius presence interaction (Table 2.1A, 2.1B), that showed that the number of surviving thrips was related both to the initial infestation and to the presence of Orius (Figure 2.1A, 2.1B). For *O. laevigatus*, there was also an effect of the plant species used (Table 2.1A) that indicated a slightly better thrips control on green bean (Figure 1B), whereas the plant factor was not significant for *O. majusculus* (Table 2.1B).

Table 2.1: Summary of GLM results on the effects of plant species, thrips density and *Orius* spp. presence on the survival of *F. occidentalis* nymphs after 24 h confined with (A) *O. majusculus* and (B) *O. laevigatus*.

Source of variation		df	MSE	*F*	*P*
(A)	Plant species	1	0.0514	0.13	0.7159
	Thrips density	1	237.3441	613.49	<0.0001
	Orius presence	1	178.1788	460.56	<0.0001
	Plant species x thrips density	1	0.0165	0.04	0.8365
	Plant species x *Orius* presence	1	0.3719	0.96	0.3284
	Thrips density x *Orius* presence	1	22.4217	57.96	<0.0001
	Plant species x thrips density x *Orius*	1	0.1449	0.37	0.5415
	Error	152	0.3868		
(B)	Plant species	1	3.3980	13.23	0.0004
	Thrips density	1	168.8287	657.12	<0.0001
	Orius presence	1	228.2179	888.27	<0.0001
	Plant species x thrips density	1	0.7989	3.11	0.0799
	Plant species x *Orius* presence	1	0.3287	1.28	0.2598
	Thrips density x *Orius* presence	1	13.6753	53.23	<0.0001
	Plant species x thrips density x *Orius*	1	0.1532	0.60	0.4411
	Error	152	0.2569		

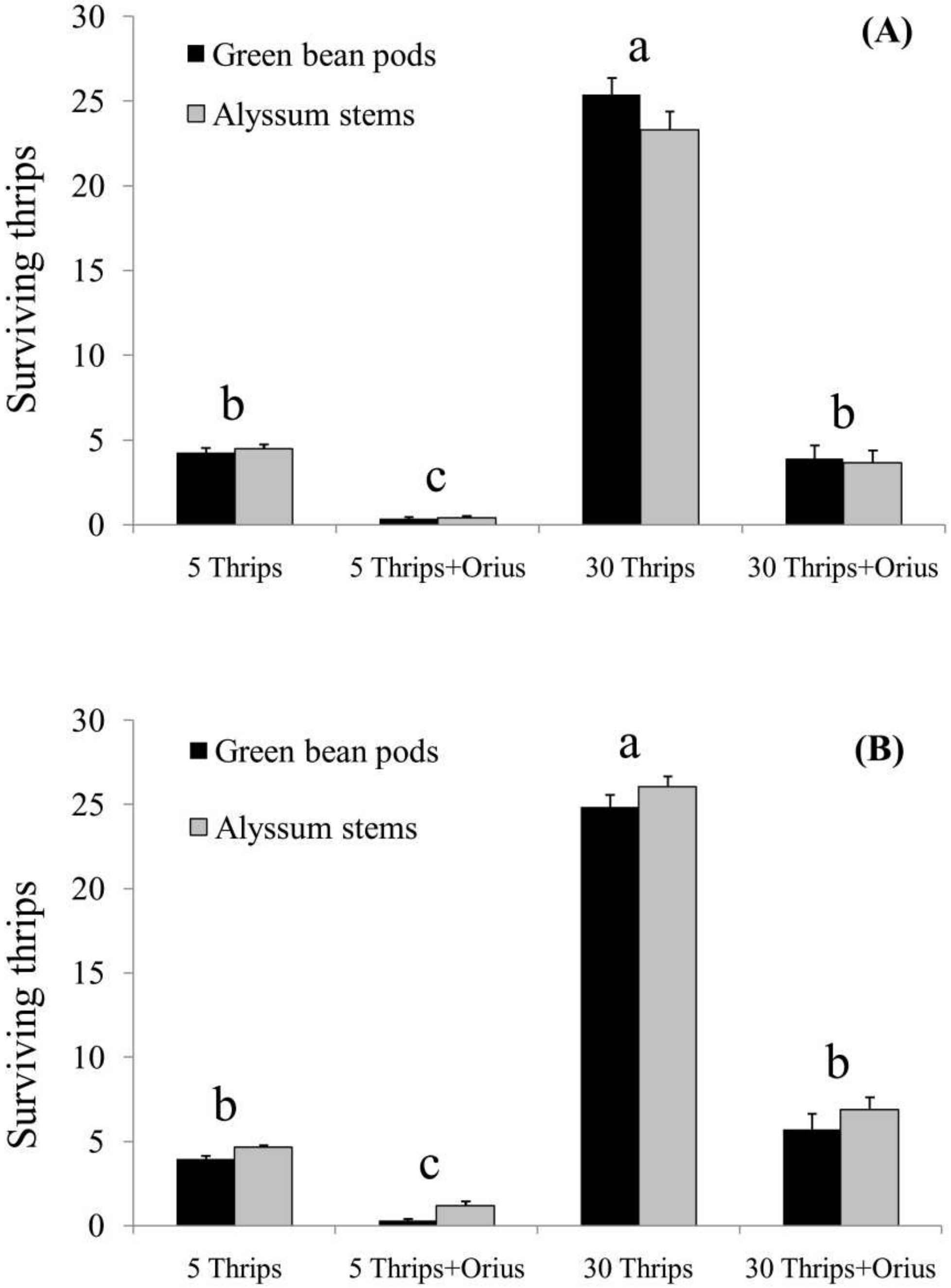


Figure 2.1: Mean number (SEM) of second instar *F. occidentalis* surviving after a 24 h exposure of 5 or 30 thrips with or without *O. majusculus* (**A**) or *O. laevigatus* (**B**) female on a flowering alyssum stem or green bean pod (n=20).

3.2 Control of thrips population growth on alyssum plants by *O. majusculus*

The highest number of thrips nymphs was found on both treatments with-thrips and without-predator ($F_{6,\ 63}$= 2.89, *P*= 0.0149), and there were no differences between all other treatments (Figure 2.2). The number of recorded thrips adults was much lower than that of the nymphs. A higher number of thrips adults was also found on both treatments with-thrips and without-predators ($F_{6,\ 63}$= 3.56, *P*= 0.0042) (Figure 2.2). Thrips were also recovered from the cages where they had not been released, but overall the smallest thrips populations were found in the treatments with the presence of predator. In regards to Orius establishment (Figure 2.3), significantly more *O. majusculus* nymphs were found when *E. kuehniella* eggs had been added as prey than in any other treatment ($F_{6,\ 63}$= 34.53, *P*<0.0001). No significant differences were recorded between all other treatments with Orius. No Orius were found in control plants, but few nymphs were found on the thrips treatments where the predator had not been released (Figure 2.3). Some mites belonging to the predatory genus *Eugamasus* spp. were found in all treatments but the numbers found were not statistically different among them ($F_{6,\ 63}$= 1.92, *P*= 0.09).

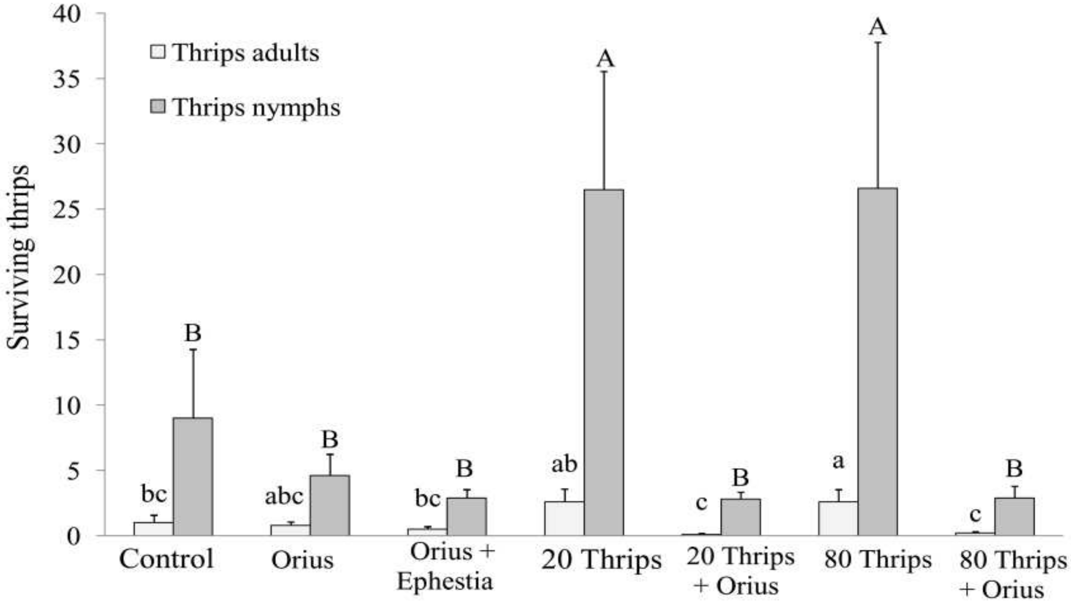


Figure 2.2: Mean number (SEM) of thrips adults and nymphs surviving after a period of 8 days on different thrips and Orius treatments on a flowering alyssum stem. Higher and lower case letters indicate thrips nymphs and adults numbers differences among treatments, respectively ($P < 0.05$).

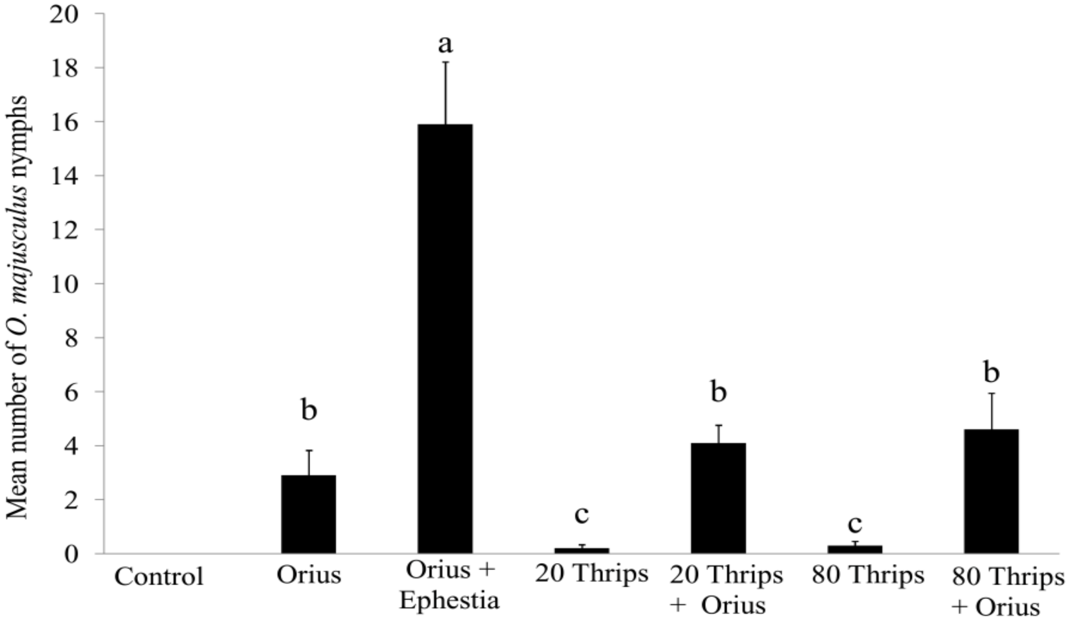


Figure 2.3: Mean number (SEM) of *O. majusculus* nymphs after a period of 8 days on different treatments.

3.3 Suppression of thrips population growth by *O. majusculus* in semi-field conditions

The weekly samplings of thrips in alyssum in the first experiment showed that there was not a treatment effect ($F_{1,\ 6}$= 3.93, *P*= 0.0948) but there was both a significant time ($F_{8,\ 48}$= 31.46, *P*<0.0001) and a treatment x time interaction effect ($F_{8,\ 48}$= 7.28, *P*= 0.0014). Lower numbers of thrips were observed throughout the experiment in the high Orius density cages, although a significant difference was only found two weeks at the end of the trial (Figure 2.4). Overall, the average Orius-day populations on alyssum were significantly different between both Orius cage treatments (23.63 ± 7.13 and 0.00 ± 0.0 respectively; t = 3.32, *P* = 0.04). However, although the additional intensive Orius samples on alyssum at the end of the experiment indicate a high establishment of Orius in the high Orius cages (Table 2.2), this was mainly restricted to one cage and the mean number of Orius was not significantly different from the low Orius density cages (t= 1.59; *P*= 0.16). A similar pattern was obtained on lettuce plants, and mean numbers of Orius and thrips were not significantly different between both treatments, neither during the experiment (t= 1.46; *P*= 0.19 and t= 0.65; *P*= 0.55 respectively; Table 2.2) nor at the additional intensive Orius samples (t= 2.07; *P*= 0.08).

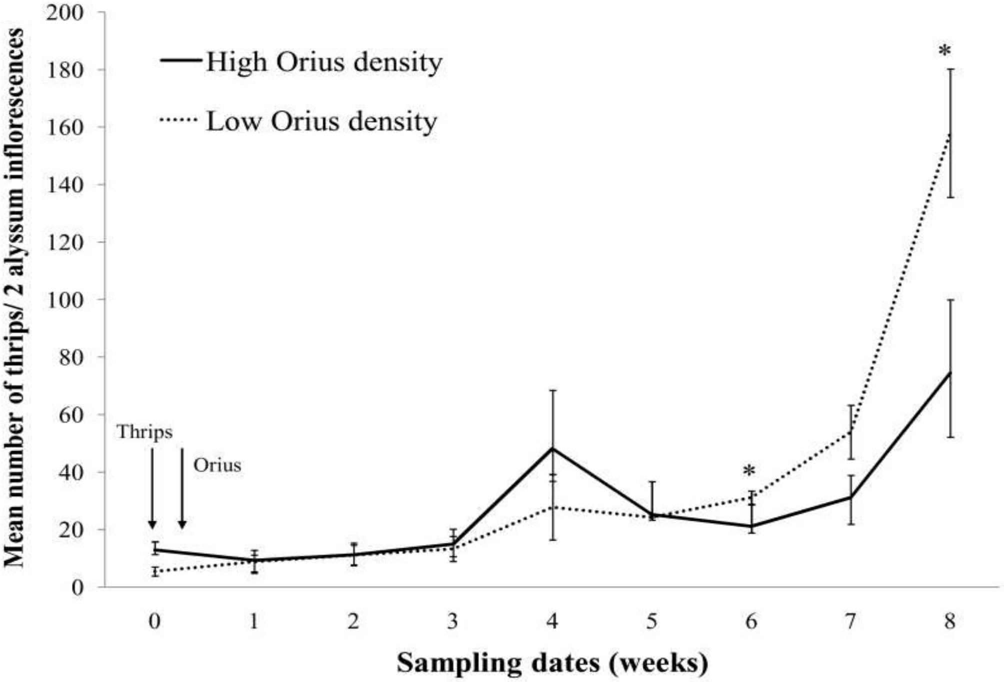


Figure 2.4: Weekly counts of thrips (mean ± SE) on alyssum plants at low and high *O. majusculus* density in the first greenhouse experiment. Asterisks indicate differences among treatments ($P < 0.05$).

Table 2.2: Mean (± SE) numbers of *O. majusculus* and *F. occidentalis* (nymphs and adults) on alyssum and lettuce in greenhouse experiment 1.

	Lettuce sampling		Additional lettuce sampling	Additional alyssum sampling
	Thrips	Orius	Orius	Orius
High Orius density	4.67 ± 2.72	8.00 ± 7.51	5.33 ± 3.53	17.67 ± 15.24
Low Orius density	6.6 ± 1.69 ^{ns}	0 ± 0 ^{ns}	0 ± 0 ^{ns}	0 ± 0 ^{ns}

ns within a column indicate non-significant differences between both Orius density treatments

Analogously, the weekly samplings of thrips in alyssum in the second experiment showed that there was not a treatment effect ($F_{1,5}$= 5.57, *P*= 0.0648), but that there was both a significant time ($F_{6,30}$= 9.51, *P*<0.0001) and a treatment x time interaction ($F_{6,30}$= 3.53, *P*= 0.0092). Lower numbers of thrips were observed throughout the experiment in the high Orius density cages although a significant difference was only found in one week (Figure 2.5). Overall, the average Orius-day populations were significantly different between both Orius cage treatments (17.72 ± 0.97 and 1.63±0.27 respectively; t = 3.45, P < 0.001), although the additional intensive Orius samples on alyssum at the end of the experiment show similar establishment of Orius in both Orius density cages (t= 0.23; *P*= 0.82) (Table 2.3). On lettuce plants, similar numbers of Orius and thrips were found in both treatments (t= 1.63; *P*= 0.15 and t= 0.54; *P*= 0.61, respectively; Table 2.3). The additional intensive Orius samples on lettuce also did not show differences among treatments (t= 1.54; *P*= 0.18).

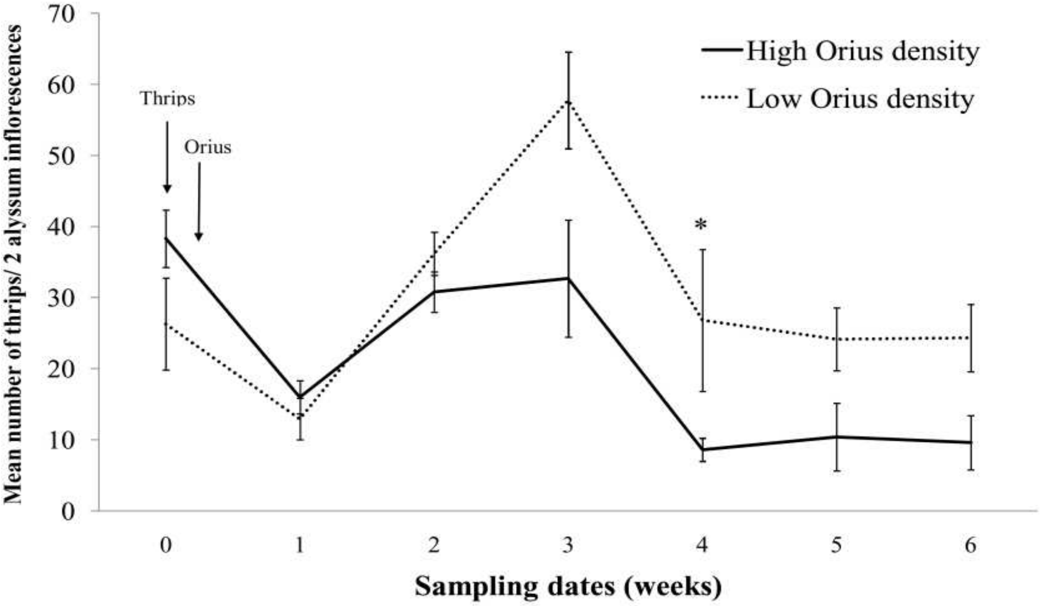


Figure 2.5: Weekly counts of thrips (mean ± SE) on alyssum plants at low and high *O. majusculus* density in the second greenhouse experiment. Asterisks indicate differences among treatments ($P <$ 0.05).

Table 2.3: Mean (± SE) numbers of *O. majusculus* and *F. occidentalis* (nymphs and adults) on alyssum and lettuce in greenhouse experiment 2.

	Lettuce sampling		Additional lettuce sampling	Additional alyssum sampling
	Thrips	Orius	Orius	Orius
High Orius density	19.75 ±5.25	2.5 ± 0.96	4.5 ± 1.7	2.25 ± 1.43
Low Orius density	23.25 ± 3.90 ^{ns}	0.75 ± 0.48 ^{ns}	1.25 ± 1.25 ^{ns}	2.75 ± 1.60 ^{ns}

ns within a column indicate non-significant differences between both Orius density treatments

4. Discussion

In recent years there has been an increasing interest in the use of insectary plants for conserving beneficial insects in crops. However, the control of pests that share the same plant with the predators is not well studied. The current work was designed to evaluate to what extent *Orius* spp. is able to control its main prey, thrips, on alyssum. A good control of thrips by *Orius* spp. was obtained in the experiments carried out in the lab. However, the results of the greenhouse experiments showed that, although thrips numbers were lower in the high Orius density cages, good thrips control was not achieved.

The presence of both *O. majusculus* and *O. laevigatus* significantly reduced the thrips numbers at both densities, either on alyssum stems or on green bean pods. Other authors have shown the effectiveness of both Orius species preying on thrips on green bean pods (Riudavets and Castane, 1998; Tommasini *et al.*, 2004). Monserrat *et al.* (2000) also found that these two predatory bugs were efficient at consuming thrips on cucumber leaf discs at densities that varied between 1 and 30 second instar larvae, but for a shorter period of time (6 h). Our results obtained for green bean pods and alyssum flowering stems are similar and demonstrate that the presence of other resources (pollen and nectar in alyssum flowers) did not reduce predation on thrips with *O. majusculus* while it was slightly reduced with *O. laevigatus*. This is a remarkable result because it has been shown that pollen sometimes can increase thrips populations (Hulshof *et al.*, 2003) or diminishes predatory ability of *Orius* spp. (Skirvin *et al.*, 2007) and of other predatory genus, too (Spellman *et al.*, 2006). Both species are able to successfully locate and capture thrips on alyssum, however, the effects of the plant on the predatory activity were different for each species. The efficacy of *Orius laevigatus* reducing thrips numbers was affected by the plant species, with a lower reduction of thrips on alyssum compared with green bean pods, while *O. majusculus* was not affected by plant species. Other authors have suggested that plants influence the Orius- thrips interactions (Brown *et al.*, 1999) or that predation rates can be diminished in more complex environments such as entire plants or flowers (Coll and Ridgway, 1995; Baez *et al.*, 2004). This could have been what happened with *O. laevigatus*, with the green bean pods representing a much more simple structure compared to alyssum stems, and thus making it easier to search for and locate thrips. However, the efficacy of *O. majusculus* to reduce thrips numbers was not affected by the plant species. Therefore *O. majusculus* may represent a better option to be used in conservation biological control programs together with alyssum plants.

In the second experiment, we could see that alyssum is a good reproductive host for thrips, with significantly more nymphs found on both treatments where thrips but no Orius had been released. When *O. majusculus* and *F. occidentalis* were confined on alyssum plants for a longer period of time, the predator significantly suppressed thrips populations at both thrips densities compared to the treatment without Orius. Other authors have also found that *O. majusculus* effectively preys on *F. occidentalis*, either in the lab (Riudavets and castanet, 1998; Montserrat *et al.,* 2000) or in the field (Blaeser *et al.*, 2004; Fitzgerald, 2006). *Orius majusculus* also reproduced and established on all treatments where it had been released, but showed much better performance when fed with *E. kuehniella* eggs than when preying on a growing population of *F. occidentalis*. Other authors have also shown that thrips are a less suitable diet than Lepidopteran eggs for *O. majusculus* and other Orius species (Kiman and Yeargan, 1985; Tommasini *et al.*, 2004). Lepidopteran eggs are known to provide a high quality diet for predators (Cohen, 1989; Eubanks and Denno, 1999). Likewise previous work showed, as expected, that fecundity was much higher on alyssum with *E. kuehniella* eggs as prey compared with only alyssum plants (Pumariño and Alomar, Submitted). In the treatments with thrips, *O. majusculus* successfully reproduced and established too, despite the number of nymphs obtained was smaller. According to our results, thrips support *O. majusculus* reproduction on alyssum besides the nutrition this prey provides does not seem to be very different from the plant itself. Similarly, the total fecundity obtained for *O. insidiosus* fed on pollen was not different from the fecundity obtained on a diet of thrips (Kiman and Yeargan, 1985).There were no differences between the number of predator nymphs obtained in both treatments where thrips had been introduced and the only Orius treatment where thrips had not been released. This is not surprising due to contaminations by thrips as shown in the control treatment, and similar numbers of thrips recovered in the three treatments. Some *O. majusculus* nymphs were found in the two treatments with thrips, where the predator had not been introduced, however the number found was negligible (in total 4 nymphs were found throughout the experiment). This experiment shows that *O. majusculus* feeds on *F. occidentalis* when they inhabit together and moreover that the predator successfully controls the pest and establishes in alyssum.

The good control of thrips was not achieved in semi-field conditions, where the thrips populations were not statistically different for most weeks between both Orius treatments. Notwithstanding, the numbers of thrips obtained were lower in the high Orius density treatment in both experiments. *Orius majusculus* has been shown to reduce thrips populations in greenhouse crops such as strawberry (Fitzgerald, 2006), cucumber and pepper (Fischer *et al.*, 1992), as well as in ornamental plants such as *Saintpaulia ionantha* (Blaeser *et al.*, 2004). These results contrast with the ones obtained in the two previous experiments, where the predators clearly reduced the numbers of thrips. Nevertheless, *O. majusculus* successfully reproduced in the alyssum plants and nymphs were found throughout both experiments. The low establishment of *O. majusculus* could be related with the fact that maybe thrips are not the most suitable food source for the predator, as it was found in the experiment carried out in microcosm conditions. We hypothesize that a possible solution to achieve a faster *O. majusculus* establishment could be the addition to the crops of some highly nutritious prey such as *E. kuehniella* eggs. The lettuce samplings showed that *O. majusculus* dispersed to the crop in the high Orius density treatments in both experiments, and also in the low Orius density treatment of the second experiment. Some more predators were found on lettuce in the high Orius density treatment in the second experiment. A few thrips were found in the lettuce samplings, but they were seen equally in both treatments and experiments and no incidences of any virus infestation were observed on the lettuce plants. Despite the fact that less thrips were found in the treatments with high Orius density, they were not statistically different from the treatments with low Orius density and thus, the results observed in these semi-field experiments are not very conclusive and further research is needed.

In summary, our results demonstrate that *O. majusculus* and *O. laevigatus* can effectively reduce thrips populations on alyssum on microcosm conditions. Furthermore, *O. majusculus* can successfully reproduce and develop with *F. occidentalis* as prey on alyssum in these same microcosm conditions. However in semi-field conditions, despite the fact that the thrips numbers were lower with high Orius densities, this predator did not significantly reduced the pests populations. As a result, further research need to be done in semi-field or field conditions in order to achieve more conclusive results about the ability of *O. majusculus* to control thrips populations on alyssum. Notwithstanding, alyssum seems a good companion plant for the *Orius* spp. conservation in the field: it allows the predator reproduction and establishment without enhancing pest populations.

Acknowledgements

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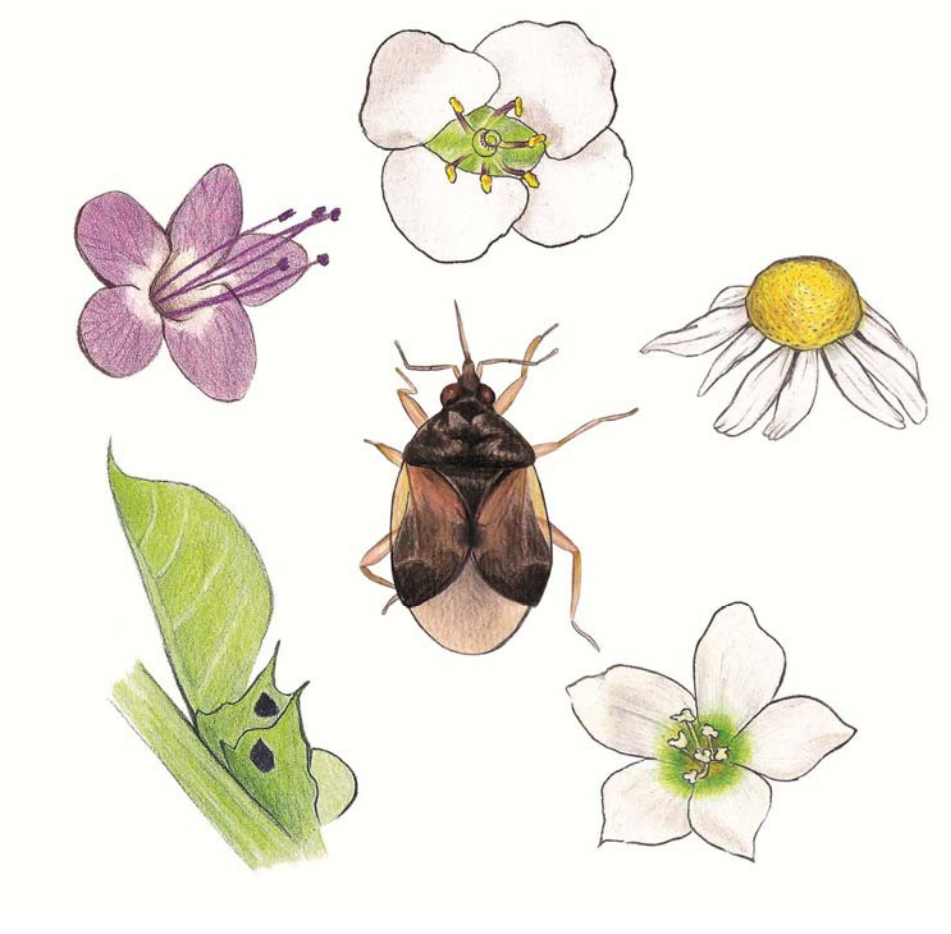
Diversity of floral and extrafloral nutritional resources on the fitness of an omnivorous bug, *Orius insidiosus* (Hemiptera: Anthocoridae)



Lorena Pumariño, Oscar Alomar and Jonathan G. Lundgren

This chapter has been submitted to Journal of Insect Conservation

Front page: *Orius* spp., alyssum, buckwheat, phacelia and chamomile flowers. Faba bean stem.

Resumen

La manipulación del hábitat y el incremento de la biodiversidad son importantes herramientas para la mejora del control biológico de plagas, pero es también relevante evaluar los beneficios aportados por cada especie vegetal cuando se diseñan dichas estrategias de control biológico por conservación. *Orius insidiosus* es un importante depredador de trips y pulgones que a su vez se alimenta de tejidos vegetales, lo cual es muy importante en programas de control biológico por conservación. A pesar de la relevancia de este depredador, poco se sabe acerca de los efectos que los distintos tejidos vegetales ejercen sobre su reproducción y supervivencia así como su estado nutricional. En este trabajo se han estudiado cinco muy distintas especies vegetales, el hecho de restringir o no el acceso al polen y /o néctar y cómo un incremento de la biodiversidad afecta a la fecundidad, supervivencia y estado nutricional de *O. insidiosus*. Dicho depredador obtuvo muy variados beneficios dependiendo de las distintas especies vegetales, y a su vez esto se debió en parte a la disponibilidad o no de acceso al polen y el néctar. El aumento de la diversidad no generó un aumento de la fecundidad, sin embargo la planta dónde el número de huevos puestos fue menor cuándo las plantas se ofrecieron de manera individual, fue la planta con mayor número de huevos puestos cuando las plantas se ofrecieron de manera conjunta. En conclusión, los beneficios que *O. insidiosus* obtiene son distintos según la especie vegetal usada y además un aumento de la diversidad vegetal puede provocar efectos impredecibles y beneficiosos en la salud de dicha especie.

Abstract

Habitat manipulation and increasing biodiversity are important tools for enhancing biological control of pests but it is important to evaluate the relative benefits of specific plant species when designing conservation plans. *Orius insidiosus* is an important predator of thrips and aphids that also feeds on plants that is the target of conservation biological control programs. Despite its relevance, little is known about the effects of plant subsidies on its performance or nutritional status. Here we examined the influence of five plant species (alyssum, buckwheat, phacelia, faba bean and chamomile), the effects of restricting their pollen and nectar resources, and how increasing plant diversity affects *O. insidiosus* fecundity, survival and nutritional status. Plant species varied in their suitability for *O. insidiosus*, which was driven in part by the availability of the pollen or nectar sources. Offering plants as a mixture did not improve fecundity, however the plant least preferred for oviposition under no-choice tests (faba bean) became the preferred egg-laying site when the plants were offered in combination. We conclude that the benefits obtained by *O. insidiosus* vary among plant species, and that increasing plant diversity can have unpredicted, positive effects on insect fitness.

1. Introduction

Increasing biodiversity in natural and managed ecosystems can increase pressure on herbivores and improve pest management through biological control (Andow, 1991; Landis *et al.*, 2000; Gurr *et al.*, 2003; Lundgren *et al.*, 2009). Natural enemies could be augmented through either an improvement in their fitness or an increase in their oviposition rate (Alomar and Wiedenmann, 1996; Lundgren, 2009). Fiedler and Landis (2007a, b) suggested that a viable strategy to conserve natural enemies within a habitat would be to mix plant species such that floral resources would be available over a long period of time. The most beneficial plants species should be identified and provided, either alone or in combination, in order to selectively enhance the abundance of each natural enemy under study. It is important to determine the extent to which an increase in biodiversity affects beneficial arthropods.

Plant resources (pollen, floral and extrafloral nectar or plant sap) are used by natural enemies as food sources (Wäckers, 2005; Lundgren, 2009). As a result, the provision of flowering plants can enhance effectiveness of natural enemies by increasing their longevity, fecundity, and predation or parasitism rates (Berndt and Wratten, 2005; Begum *et al.*, 2006; Lee and Heimpel, 2008). Specifically, sugar feeding improves the fitness and performance and affects the nutrient contents levels in parasitoids (Olson *et al.*, 2000; Lee *et al.*, 2004; Chen and Fadamiro, 2006; Nafziger and Fadamiro, 2011) and predators (Lundgren, 2011; Seagraves *et al.*, 2011). Research has long supported the importance of nectar feeding for parasitoid life histories, but nectar resources for predators have received less attention until recently. The impact of different pollen and nectar sources on predator survival, longevity or development should be quantified as insect conservation programs are developed for specific natural enemies.

Several factors affect the relative preferences of natural enemies for specific plant species, of which accessibility and quality of the nectar and pollen are particularly important (Colley and Luna, 2000; Ambrosino *et al.*, 2006; Hogg *et al.*, 2011). Floral architecture restricts which insects can access the floral rewards of the plant (Idris and Grafius, 1995; Patt *et al.*, 1997; Vattala *et al.*, 2006). For instance, a study has found that the numbers of insects visiting plants were lower on flowers with longer and narrower corollas (Stang *et al.*, 2006). Studies on the composition of nectar have shown that it is mainly composed by sucrose, glucose and fructose (Wäckers, 2001; Petanidou, 2005) and it usually contains low levels of amino acids (Baker and Baker, 1973). However, nectar can also contain compounds that are either toxic or repellent to flower visitors (Adler, 2000; Wäckers, 2001). A major difference between floral and extrafloral nectaries comes in their ecological roles: floral nectar is intended to attract pollinators and extrafloral nectar is used to attract natural enemies of herbivores (Lundgren, 2009). As such, extrafloral nectaries are often more exposed to potential visitors and their number and quantity of nectar may increase in response to herbivory. Despite the fact that plants are known to be important food sources for predators, the physiological benefits and metabolic uses of the different plant subsidies are still poorly understood. Some authors have observed that restricting the access of predators to nectar can reduce their survival and fitness considerably (Lundgren and Seagraves, 2011; Portillo *et al.*, 2012).

Orius insidiosus Say (Hemiptera: Anthocoridae) is an important native predator of pests in North America. It consumes various pests such as thrips, aphids and eggs of Lepidoptera (Phoofolo *et al.*, 2001; Baez *et al.*, 2004; Rutledge and O'Neil, 2005; Harwood *et al.*, 2009; Seagraves and Yeargan, 2009). Although *O. insidiosus* and congeners mainly feed on prey, these are omnivorous predators that also feed on different plant resources such as plant sap (Kiman and Yeargan, 1985; Coll, 1996; Richards and Schmidt, 1996a; Lundgren *et al.*, 2008), pollen (Kiman and Yeargan, 1985; Richards and Schmidt, 1996a; Corey *et al.*, 1998) and nectar (Yokoyama, 1978; Bugg, 1987) from several plant species. However, as far as we know, there are no data available about how nectar and pollen affect the nutrient status and performance of *O. insidiosus*.

The main objectives of this work were to determine (1) how plants that varied in their accessibility to the pollen and nectar affect *O. insidiosus* performance, and (2) whether increasing plant diversity provide additive or synergistic effects on *O. insidiosus* performance.

2. Material and methods

2.1 Sources of insects and plants

Orius insidiosus adults were purchased from Koppert Canada Limited (Scarborough, ON, Canada). Upon arrival they were maintained in vermiculite on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (Beneficial Insectary, Redding, CA), and green bean (*Phaseolus vulgaris* L.) pods from a grocery store were used as a water source. Adults were kept in climatic chambers at 25 ± 1° C, 70 ± 10 % r.h. and 16: 8 L: D photoperiod until the experiments.

The plant species used were alyssum (*Lobularia maritima* L. cv. Carpet of Snow), buckwheat (*Fagopyrum esculentum* Moench cv. Mancan), phacelia (*Phacelia tanacetifolia* Bentham), faba bean (*Vicia faba* L. cv. Negreta) and chamomile (*Matricaria chamomilla* L.).

Alyssum plants were obtained from a local nursery and maintained in the greenhouse until they were used in the experiments. The rest of the plants were grown in the greenhouse from seed in a compost/peat-moss/perlite mixture (4:2:1), watered daily. The plants were allowed to grow until flowering (or a height in faba bean that was equivalent to flowering chamomile, the shortest of the plants).

These plant species were selected based on their use in conservation biological control programs, and their difference in floral architecture. According to Fiedler *et al.* (2008), three of the four most studied plant species in habitat management include alyssum, buckwheat, and phacelia, and thus they were chosen for this study. Another reason these plants were chosen was that alyssum and buckwheat present a short corolla (Vattala *et al.*, 2006) while phacelia presents a deep corolla (Baggen *et al.*, 1999). Additionally, a plant with extra-floral nectares, faba bean, and a plant whose nectaries are hidden in a capitulum (Patt *et al.*, 1997), chamomile, were selected for the study.

2.2 Influence of plant sugars on *O. insidiosus* performance.

Potted alyssum, buckwheat, phacelia, chamomile and faba bean plants from the greenhouse were used in the experiment. Stems from those potted plants and a water saturated cotton wick were enclosed within a plastic cup (710 ml; Solo, Urbana, IL, USA) which was sealed with adhesive clay (Loctite®, Henkel Corporation, Avon, OH, 44011). The top of the arena was replaced with cloth mesh to allow ventilation. There were two sub-treatments for each plant species, either (1) the flowers (including pollen and floral nectar) or extra floral nectaries were covered with Parafilm® (Pechiney Plastic Packaging, Menasha, WI, 54952) (from now on termed the "covered treatment") and (2) the flowers or extra floral nectaries were exposed to the insects (from now on termed the "uncovered treatment"). In the uncovered treatment, a strip of Parafilm® was wrapped around the stem to standardize the experimental conditions. Care was taken to provide similar surface areas of all the plant species in the individual treatments. Two *O. insidiosus* females (2-6 d old) were randomly assigned to each treatment for 48 h. After this exposure, the number of *O. insidiosus* females alive, dead or missing was recorded and the number of eggs laid was counted microscopically. If only one *O. insidiosus* survived the exposure, it was frozen in a microcentrifuge tube at -20° C for post-mortem nutrient (glycogen and lipids) analyses. If two *O. insidiosus* females were found alive, the first one was kept for the nutrient analyses (described below) and the fecundity and survival of the second one was examined. To determine if prior treatment had any influence on the fecundity and longevity of *O. insidiosus*, this second female was placed in a Petri dish with a green bean pod and a saturated cotton wick; these arenas were checked daily for survival and for the final number of eggs laid. Two other treatments received only water (in form of a water saturated cotton wick) or a green bean pod with *ad libitum E. kuehniella* eggs affixed to a Post-it® (≈1cm²; 3M St Paul, MN) also during 2 d. Additional females from the colony were directly taken out and subjected to the nutrient analyses too. Environmental conditions of the experiment were the same as in the rearing.

2.3 Glycogen and lipids assays

Glycogen and lipids were quantified using colorimetric assays modified from methods described by Van Handel (1985a, b). Before the analysis, the bugs were rinsed in water to remove any sugar contamination. Then each insect was individually placed into a sterile microcentrifuge tube and ground in 300 µl of methanol-chloroform (a 2:1 solution) using a sterile plastic pestle. The samples were centrifuged at 13200 rpm for 4 min, and the supernatant was placed in a glass tube for the lipid assays; the remaining precipitate was set aside for the glycogen assay. For the analysis of the lipids, the solvent was evaporated at 90º C. Sulfuric acid (40 µl) was added and the solution was heated for 2 min at 90ºC. After cooling the samples, they were incubated in 960 µl of vanillin-phosphoric acid reagent (600 mg vanillin in 100 ml water, diluted in 400 ml of 85% phosphoric acid; vanillin product #V10-100; Fisher Chemical, New Jersey) for 25 min at 23º C. An aliquot (200 µl) of each sample was placed on a 96-well plate and optical density was read at 525 nm using a spectrophotometer (µQuant, BioTek Instruments Inc., Winooski, VT, USA). Three positive controls (25 µl of olive oil solution [109 µl extra virgin olive oil in 100 µl of chloroform]), and three negative controls (25 µl of distilled water) were subjected to the same assay procedure described above.

The glycogen content of the precipitate was quantified using an anthrone assay. One ml of anthrone reagent (750 mg anthrone in 150 ml water, diluted in 380 ml 98% concentrated sulfuric acid; anthrone product #319899, Sigma-Aldrich, St. Louis, MO) was added to each of the tubes containing the precipitate and heated at 90º C for 15 min. An aliquot (200 µl) of each sample was placed on a 96-well plate and optical density was read at 630 nm using the spectrophotometer. Three positive controls (25 µl of glycogen solution [25 mg glycogen from oyster, Type II, Sigma-Aldrich, GmbH, Japan, in 25 ml water]), and three negative controls (25 µl of distilled water) were subjected to the same procedure described above.

The lipid and glycogen content per insect was calculated from the absorbance values using a standard curve, as calculated by Olson *et al.* (2000). To generate the lipid and glycogen curves extra virgin olive oil and glycogen from oyster were used, respectively. Three replicates for each concentration level (1, 5, 10, 25, 50, 75 and 100 µg brought to a total volume of 1 ml with anthrone and vanillin reagents for glycogen and lipid standards, respectively) were prepared and nutrient amounts were calculated from the resulting linear regression equations. The mean absorbance read from the 48 h starved insects was subtracted from the experimental samples. The absorbance was then transferred to the standard curve and the glycogen and lipid contents per insect were obtained.

2.4 Influence of increasing plant diversity on *O. insidiosus* performance

A second experiment was designed to evaluate how mixtures of three plant species (versus the individual plant species) affected the fitness of *O. insidiosus*. Faba beans, chamomile, and buckwheat were produced as previously described. Severed plant stems were inserted into a 50 ml centrifuge tube filled with water, and the open top of the tube was sealed around the stem using Parafilm®. A final treatment received only water on saturated cotton wick. Similar surface areas of the three plant species in the individual treatments were used, and this was approximately the same foliage density as in the mixed plant treatment.. The severed stems in water were housed within two plastic cups (710 ml; Solo, Urbana, IL, USA) held mouth-to-mouth with Parafilm®. The plant stems were inserted through a hole in the base of the bottom cup which was sealed with adhesive clay (Loctite®, Henkel Corporation, Avon, OH, 44011). A water saturated cotton wick was also added to each cup. The top of the arena was replaced with cloth mesh to allow ventilation. Two *O. insidiosus* females, received 3 d earlier from Koppert, were placed in each arena for the duration of the experiment. Environmental conditions of the experiment were 25 ± 1° C, 40 ± 10 % r.h. and 16: 8 L: D photoperiod. There were a total of 16 cups assigned to each treatment. Survival and reproduction by the females was monitored. Survival was checked approximately every 48 h until all the bugs had died. As plants deteriorated, they were replaced with fresh ones, and the number of eggs laid on each plant species was recorded at least once per week. Because two females per cup oviposited, we standardized the number of eggs laid per "*Orius* day", that is the number of eggs laid per female per day. Any females that were lost or killed accidentally were included in the survival analysis as censored data points.

2.5 Statistical analysis

In the first experiment, the number of eggs laid per female and per plant structure, and the number of females that survived or were missing over the 48 h period of the experiment were compared among treatments using the GLM procedure (with plant species and nectar availability as main effects). The number of eggs laid and the longevity in the Petri dish with green bean pods (subsequent to the plant exposure period) were compared among treatments using the same procedure. The GLM procedure was also used to determine the effects of plant species and nectar availability on glycogen and lipid contents. The colony females, water only, and green bean pods + *E. kuehniella* eggs treatments were considered as experimental controls and were omitted from these statistical analyses examining glycogen/lipids dynamics. The GLM analyses did analyze the plant species and nectar availability factors and the control treatments could not be included in such analyses. Significantly different means were separated with the Duncan test ($P < 0.05$). Prior to the analysis the number of eggs laid on green bean and the glycogen contents were square root transformed.

In the second experiment, survival was compared among treatments using a Kaplan-Meier non-parametric survival analysis. For this analysis, each female was regarded as an experimental unit. Subsequently, after we omitted the control, an ANOVA was used to determine differences among treatments. The total number of eggs laid per treatment and the number of eggs laid per "*Orius* day" were compared among treatments with independent ANOVAs. Means were separated with the Duncan test ($P < 0.05$). Prior to the analysis the total number of eggs in the overall experiment was log+1 transformed and the total number of eggs laid per plant species and the number of eggs laid per female within the mixed treatment were square root transformed. All the analyses were conducted using SAS Enterpreise Guide 4.2.

3. Results

3.1 Influence of plant sugars on *O. insidiosus* performance.

3.1.1 Survival and longevity.

A higher number of females survived during the first 48 h of the experiment on phacelia than on buckwheat or chamomile plants ($F_{4, 266} = 2.75$, $P = 0.03$) (Table 3.1). Females that were on plants with the nectar/pollen uncovered had higher survival during these 48 h ($F_{1, 266} = 6.92$, $P = 0.01$). There was no interaction between plant species and nectar availability on survival ($F_{4, 266} = 1.29$, $P = 0.27$). The percentage of females that were missing was smaller on plants with the nectar/pollen uncovered ($F_{1, 266} = 17.07$, $P < 0.0001$). However there were no effects of the plant species used ($F_{4, 266} = 1.24$, $P = 0.30$) or interactions between both factors ($F_{4, 266} = 1.20$, $P = 0.31$) (Table 3.1). The longevity of females after the 48 h plant-exposure period was similar among plant species, although the availability of nectar/pollen increased female longevity over treatments without these resources (plant species: $F_{4, 84} = 1.03$, $P = 0.40$; nectar/pollen covered or exposed: $F_{1, 84} = 3.90$, $P = 0.05$; interaction: $F_{4, 84} = 1.79$, $P = 0.14$) (Table 3.2).

Table 3.1: Percentages of survivor and missing *Orius insidiosus* females in the first experiment. Sample sizes are indicated in parentheses. Covered treatments had their flowers or extrafloral nectaries covered with Parafilm, and the uncovered treatments had their resources exposed (a piece of Parafilm was placed randomly on the plants).

	% Survival females		% Missing females	
	Covered	Uncovered	Covered	Uncovered
Chamomile	31.67 (30)	50.00 (31)^{b}	35.00	14.52
Phacelia	63.16 (19)	68.42 (19)^{a}	21.05	5.26*
Buckwheat	30.30 (33)	53.13 (32)^{b,*}	25.76	15.63
Alyssum	39.13 (23)	62.00 (25)^{ab,*}	39.13	12.00*
Faba bean	53.13 (32)	48.44 (32)^{ab}	20.31	15.63

Differences among plants (within a column) are indicated by lower case letters; asterisks indicate significant differences between columns within a plant species (α=0.05).

Table 3.2: *Orius insidiosus* females mean longevity (± SEM) in the first experiment. Sample sizes are indicated in parentheses. Covered treatments had their flowers or extrafloral nectaries covered with Parafilm, and the uncovered treatments had their resources exposed (a piece of Parafilm was placed randomly on the plants).

	Longevity on bean (days)	
	Uncovered	Covered
Chamomile	2.00 ± 0.35 (4)	2.35 ± 0.30 (14)
Phacelia	1.78 ± 0.28 (9)	2.11 ± 0.54 (9)
Buckwheat	2.33 ± 0.61 (6)	1.83 ± 0.30 (12)
Alyssum	1.33 ± 0.33 (6)	3.00±0.44 (12)
Faba bean	1.50 ± 0.19 (12)	2.11 ± 0.39 (9)

3.1.2 Fecundity.

During the 48 h exposure period, the availability of floral and extrafloral resources affected the number of eggs laid ($F_{1,266} = 6.32$, $P = 0.01$), but in pairwise contrasts, the difference was only significant for chamomile plants: more eggs were laid on uncovered than on covered chamomile plants. Some aphids were found to accidentally occur on the chamomile flowers and this likely increased the number of eggs laid on this treatment. The highest number of eggs was laid on chamomile and phacelia, and those numbers were statistically higher than on faba bean and alyssum ($F_{4,266} = 4.22$, $P = 0.003$). There was no interaction between plant species and nectar availability on fecundity during this period ($F_{4,266} = 3.35$, $P = 0.01$) (Figure 3.1a).

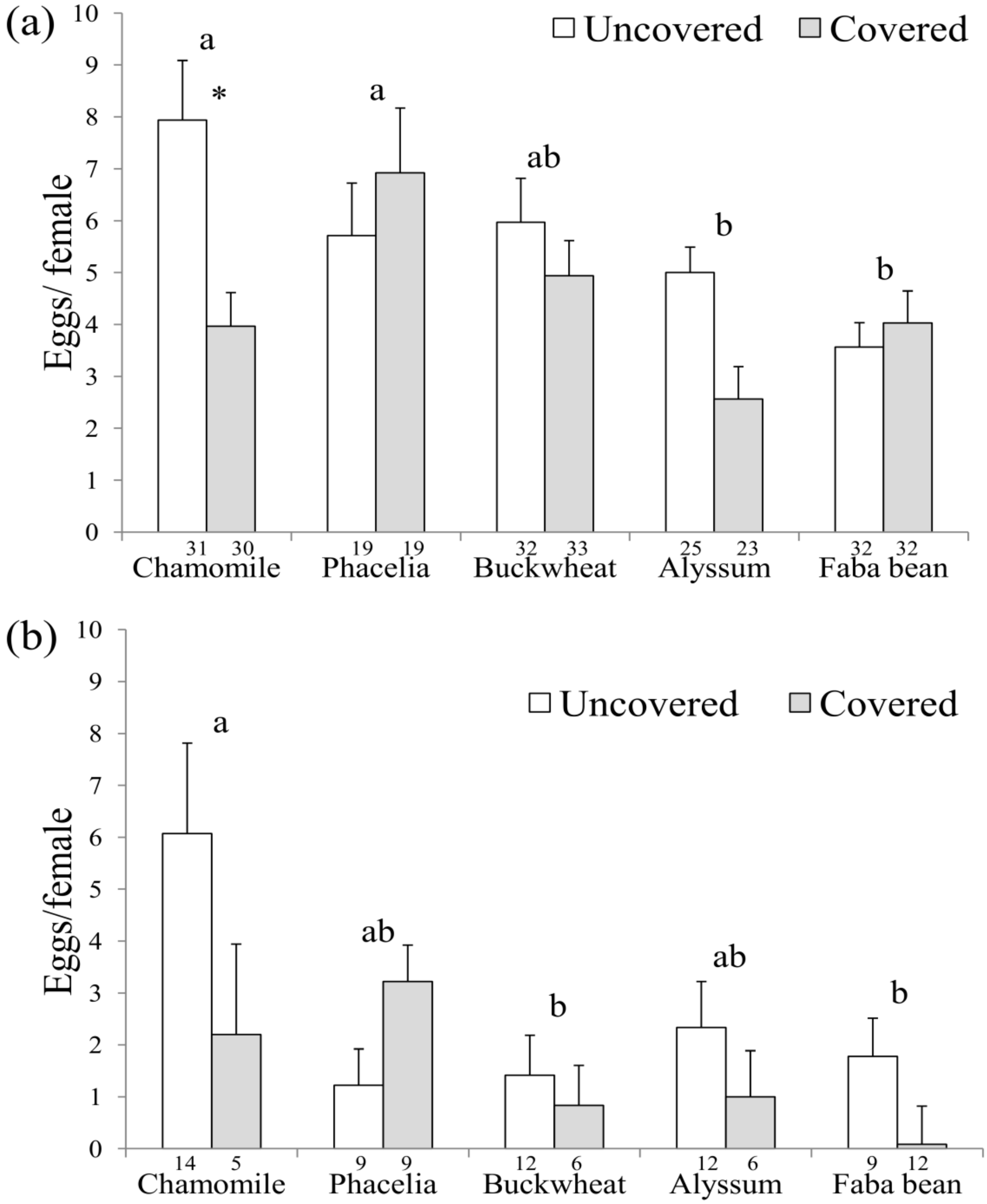


Figure 3.1a: Mean (SEM) number of eggs laid per *Orius insidiosus* female during the 48 h exposure period. Plants in the “covered” subtreatments had their floral or extrafloral resources covered with Parafilm. **3.1b**: Mean (SEM) number of eggs laid per female after a 48 h exposure to each plant species. During this phase of the experiment, females were allowed to lay eggs into green bean pods. Numbers of replicates are shown on the x axis.

After the 48 h exposure period, the highest number of eggs laid on green bean was obtained from the females whose former plant was chamomile but there was no effect of the previous availability of floral or extrafloral resources on egg production during this stage of the experiment (plant species: $F_{4, 84} = 2.89$, $P = 0.03$; covered or uncovered: $F_{1, 84} = 2.32$; $P = 0.13$; interaction: $F_{4, 84} = 2.05$, $P = 0.10$) (Figure 3.1b).

3.1.3 Nutrient analyses.

The GLM procedure showed that the quantity of glycogen per female was higher on faba bean and alyssum than on chamomile plants ($F_{4, 159} = 7.82$, $P < 0.0001$). Covering the nectar sources did not affect glycogen content of the females ($F_{1, 159} = 0.35$, $P = 0.56$), nor was there a significant interaction between plant species and nectar availability on glycogen content ($F_{4, 159} = 0.93$, $P = 0.45$) (Figure 3.2). The mean (SEM) values of glycogen for the controls were: 3.47 ± 0.93, 0.70 ± 0.23 and 1.21 ± 0.23 µg for the colony, water and green bean pods + *E. kuehniella* females. The GLM procedure showed that the quantity of lipids per female was not different among plants ($F_{4, 163} = 2.19$, $P = 0.07$). The fact of having covered the sugar sources did not show differences among plant species ($F_{1, 163} = 0.06$, $P = 0.80$) nor was there a significant interaction between these factors ($F_{4, 163} = 0.79$, $P = 0.53$) (Table 3.2). The mean (SEM) values of glycogen for the controls were: 2.99 ± 0.48, 2.00 ± 0.54 and 2.07 ± 0.47 µg for the colony, water and green bean pods + *E. kuehniella* females.

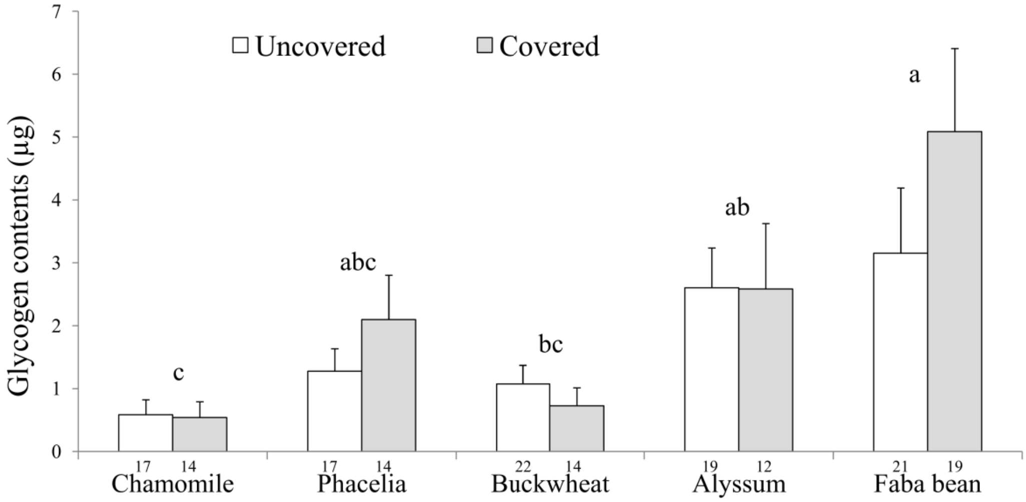


Figure 3.2: Mean (SEM) glycogen contents per *Orius insidiosus* female. Glycogen was measured using the colorimetric anthrone assay. Insects in the "covered" treatment were reared on plants with their floral or extrafloral nectaries covered in Parafilm. Numbers of replicates are shown on the x axis.

Table 3.2: Mean (SEM) lipids contents per *Orius insidiosus* female, as measured with the vanillin (lipid) reagent. The colony, water and green bean + *E. kuehniella* controls were excluded from the analyses.

Plant species		n	Lipids contents (µg)
Chamomile	Uncovered	17	1.39±0.43
	Covered	14	1.22±0.53
Phacelia	Uncovered	17	1.65±0.57
	Covered	14	2.96±0.59
Buckwheat	Uncovered	22	2.62±0.48
	Covered	14	2.67±0.66
Alyssum	Uncovered	19	2.88±0.54
	Covered	12	2.89±0.77
Faba bean	Uncovered	21	2.31±0.52
	Covered	20	1.67±0.52

3.2 Influence of increasing plant diversity on *O. insidiosus* performance

3.2.1 Survival and longevity.

The females from the four treatments had higher survival than those from the unfed control (χ^2 = 78.36, df = 4, P <0.0001). The ANOVA revealed no differences among treatments when the control was not included in the analyses ($F_{3,\,124}$ = 1.77, *P* =0.16). Mean survival times were 15.34±1.59, 16.09±1.62, 11.41±1.59, 15.50±1.64 and 3.97±0.15 d for those exposed to faba bean, buckwheat, chamomile, the mixed treatment and the control, respectively (Figure 3.3).

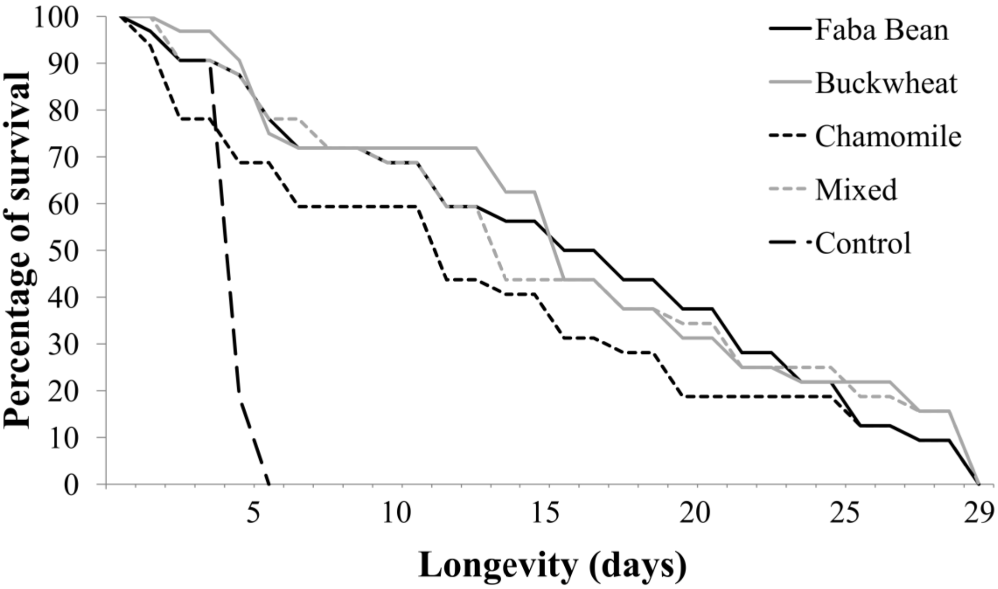


Figure 3.3: Survival probability of *Orius insidiosus* in the different treatments.

3.2.2 Fecundity.

Under no-choice conditions a higher number of total eggs ($F_{3, 60} = 6.65$, $P < 0.001$) as well as of eggs per day ($F_{3, 60} = 9.31$, $P < 0.001$) was laid on buckwheat, chamomile and the mixed treatment compared with faba bean (Figure 3.4a). However, faba bean plants were the preferred oviposition substrate in the mixed treatment for the total number of eggs laid ($F_{2, 45} = 16.02$, $P < 0.0001$) as well as the number of eggs laid per day ($F_{2, 45} = 21.40$, $P < 0.0001$) (Figure 3.4b). Under no choice conditions, the total number of eggs laid was 32.94 ± 2.94, 108.44 ± 14.01, 86.44 ± 11.66 and 114.70 ± 18.30 for the faba bean, buckwheat, chamomile and mixed treatment, respectively. When given a choice among plants, the total number of eggs laid was 68.81 ± 12.52, 36.62 ± 8.21 and 9.25 ± 2.63 for the faba bean, buckwheat and chamomile, respectively.

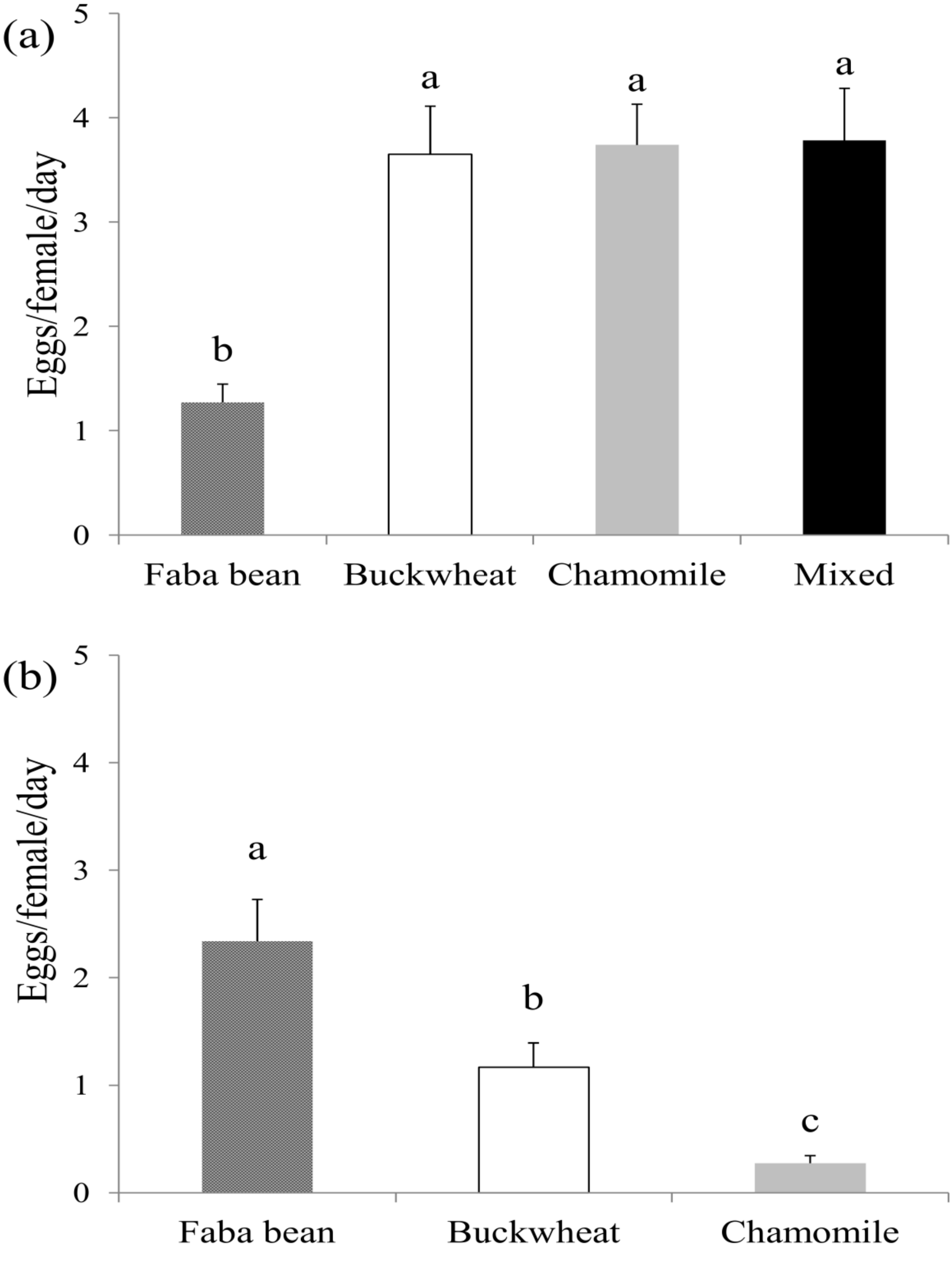


Figure 3.4a: Mean (SEM) number of eggs laid per *Orius insidiosus* female in the no-choice experiment. **3.4b**: Mean (SEM) number of eggs laid per female in the mixed treatment.

4. Discussion

Our study demonstrates that nectar and pollen from different plant species affect the fecundity, survival and nutrient status of *O. insidiosus*. Additionally, increasing plant diversity allows the omnivore to optimize their fitness by exploiting various plant-based resources such as nutrition and oviposition sites. This finding underlines the importance of choosing appropriate plants and providing plant mixtures for obtaining the best performance of predators for conservation biological control programs.

Plant species affected the survival of *O. insidiosus* females. Survival was higher on phacelia than on chamomile or buckwheat during the 48-h exposure rate. However, plant host did not influence longevity or survival once the females were moved over to green bean pods, suggesting that plant species effects were immediate, possibly stemming from nutritional or defensive variations in the plant. Irvin *et al.* (2007) studied the sugar composition of phacelia, faba bean, alyssum and buckwheat whole flowers and they determined that phacelia had more total sugars (µg) than the other flower species. It could be that the higher survival obtained on phacelia in our study is due to the fact that this plant species provides an extra source of sugar, compared with the other plants.

Plant species also affected the oviposition rate of *O. insidiosus* females; the observed patterns could have been produced by either the nutritional quality of the plant or their morphological characteristics (e.g., epidermal thickness or trichome density) that might restrict their attractiveness to *O. insidiosus*. This predatory bug laid the most eggs on chamomile and phacelia, and these numbers were statistically higher than on alyssum and faba bean. These plant effects persisted even after the females were moved onto green bean pods, but only for chamomile: the females that were reared previously on chamomile laid the highest number of eggs and the lowest were laid when faba bean and buckwheat were the host plants. There was a low number of aphids found on chamomile flowers, and we believe that this in part produced the strong ovipositional response that we saw on this species. Phacelia has been found as very attractive for some natural enemies (Ambrosino *et al.*, 2006) and our results from the no-choice tests suggests that this species could be a highly acceptable oviposition substrate for *O. insidiosus* females. Previous work has shown that the morphological characteristics of the plant also are correlated with their relative acceptance to ovipositing *O. insidiosus* (Lundgren et al., 2008). Green beans have been widely used as an acceptable oviposition substrate (Richards and Schmidt, 1996b), and so providing this substrate to all treatments was intended to separate the nutritional suitability of the different plants for oviposition from the morphological/defensive plant characteristics known to influence oviposition. The fact that we saw very similar patterns in oviposition in Figures 1a & 1b suggest that plant nutrition was a primary driver for the relative ovipositional acceptance of these plant species.

Plant species also affected levels of major storage nutrients in the females. Females on faba bean had the highest levels of glycogen, and these were higher than on buckwheat and chamomile. Glycogen and lipid contents are inversely related to egg production, and we hypothesize that this is due to for the high storage nutrient levels found in females raised on the plant species that supported the lowest oviposition rate. However, these results support our hypothesis that the extrafloral nectaries in faba bean are a very accessible source of sugar for this bug in that they were able to maintain high levels of glycogen. However, nectar alone is seldom sufficient for producing eggs, and the pollen resources found in the flowers, but not extrafloral nectaries, may have helped to support higher oviposition rates in the other plants, which depleted storage nutrient reserves. It is interesting to mention that while relative glycogen contents on faba bean were higher than the rest of the plants, the relative lipids contents were not very different from the other plant species. Although the differences in lipid contents of *O. insidiosus* reared on different plants were only marginally significant, it is remarkable that the levels obtained from the females that had been on chamomile were much lower than those obtained from the females that had been on alyssum or buckwheat. Nevertheless, this is not surprising since egg production in animals requires protein or lipids and chamomile was the plant where insects laid the most of the eggs, which could have caused the depletion of their lipid storages. These marginal differences found in lipids levels are in concordance with other works that have shown that lipids do not vary with sugar-based diets but with age in the case of two different female parasitoid species (Olson *et al.*, 2000; Lee *et al.*, 2004).

Access to floral resources or extrafloral nectar improved the survival of *O. insidiosus* females during the time they were exposed to the plants. This was particularly so for females raised on buckwheat and alyssum. Therefore it seems that facultative phytophagy by *O. insidiosus* consists not only of sucking phloem from the plant, but also includes the consumption of flower subsidies. Nectar consumption had some lasting effects on *O. insidiosus* survival after they had been removed from the plants and, although the treatment differences were only marginally significant on the longevity recorded on green bean, they suggest that females that had been previously exposed to the floral/nectar resources lived longer than those without floral resources or extrafloral nectar. The importance of nectar for *O. insidiosus* may also be supported by the fact that many of them were lost from the plants with the covered nectaries. The percentages of missing females during the first part of the experiment were higher on the covered plants (more missing on phacelia and alyssum covered than uncovered). A plausible explanation for these results could be that females on the uncovered treatments had a suitable food source available and they did not invest time in trying to escape. On the contrary, females on the covered treatments, lacking these nutritional resources may have had more incentive to find or create escape routes from the arena. In many other studies, access to sugar sources has improved the survival of predators (Lundgren and Seagraves, 2011; Portillo *et al.*, 2012) and parasitoids (Olson *et al.*, 2000; Lee *et al.*, 2004). We found that *O. insidiosus* females laid more eggs when offered nectar and pollen than when these resources were restricted on three of the four flowering plants, although the greatest benefit of floral resources was observed in chamomile which had low levels of aphids near the flowers. In contrast, covering the pollen and nectar sources did not produce different levels of glycogen or lipids in the bugs. Other recent work however has shown that while access to nectar greatly improved the levels of glycogen in a predatory beetle, the lipid contents were similar when it was compared with no access to nectar (Olson *et al.*, 2000; Lundgren and Seagraves, 2011). Access to sugar sources also increased the glycogen levels, but not the lipids on a female parasitoid species when it was compared with starved insects.

The survival curves demonstrated that longer longevities are obtained on plant diets compared with water alone. This follows the pattern of many other works that have been carried out with other predators or parasitoids (Vattala *et al.*, 2006) which demonstrate the importance of plant-based diets for the survival and thus conservation of omnivorous predators.

Offering the plant species as a mixture gave a much different interpretation of the data, especially with regard to the benefits of faba beans for *O. insidiosus* females. *Orius insidiosus* survived equally well when faba bean, buckwheat and chamomile were offered alone or in combination. These survivals contrast with those obtained in the first experiment, where the *O. insidiosus* females did not survive longer than 3 d on the green bean pods, after feeding on those plants. This emphasizes the importance of flowering plants compared with only bean pods, which have been shown to provide similar nutrition to only water (Chapter 1). The results obtained on the number of eggs laid by day and female and the total numbers of eggs laid by female were unexpected. *Orius insidiosus* laid fewer eggs on faba bean in no-choice conditions relative to all of the other treatments. However, when the three plants were offered together, *O. insidiosus* displayed the opposite pattern and faba bean was the preferred plant for oviposition. This predatory bug shows clear preferences for some plants as oviposition substrates (Coll, 1996;

Lundgren and Fergen, 2006) and plant species is even more important for oviposition site selection than prey availability (Seagraves and Lundgren, 2010). Our results show that maybe *O. insidiosus* considers faba bean a better oviposition substrate for the development of its offspring, but only when there is high quality nutrition (e.g., pollen) available for the female to mature eggs. Other work has shown that this bug changes its preference for oviposition sites on a plant species depending on whether it was offered singly or with other plants (Lundgren and Fergen, 2006), while other work shows that different combinations of plant species did not influence the proportion of eggs laid by *O. insidiosus* on each plant species (Coll, 1996) (although this study just examined small cuttings of trifoliates). Atakan (2010) found that *O. insidiosus* may prefer faba bean plants as a site for egg laying when it was compared with many other plants. In the first experiment, *O. insidiosus* females raised on faba beans with covered nectaries survived well relative to the other plant species when their flowers were covered, which is consistent with the hypothesis that vegetative structures of faba beans may have good nutrition for maintaining *O. insidiosus* survival. Although additional experiments will help elucidate these relationships, our current interpretation of our data is that faba bean could be the most suitable oviposition plant for *O. insidiosus*, but this plant can only be fully exploited when an additional protein or lipid source (either prey or pollen) accompanies it which allows females to mature eggs.

These experiments illustrate that the true conservation benefits of a plant species for a beneficial insect depend on the context in which the plants are examined. Plant species varied substantially in their ability to support survival and reproduction of *O. insidiosus* under no-choice conditions. Although faba bean did not support substantial oviposition in a no-choice situation, when it was offered with other plants it was the preferred oviposition site for *O. insidiosus* females. Our findings demonstrate that not only is it necessary to provide natural enemies with pollen or nectar sources, but that each predator will respond differently to various plant resources. As such, predicting which plants are most beneficial for conservation biological control programs under various circumstances is inherently very difficult, and it makes the case for conserving plant biodiversity as a whole in and near agroecosystems.

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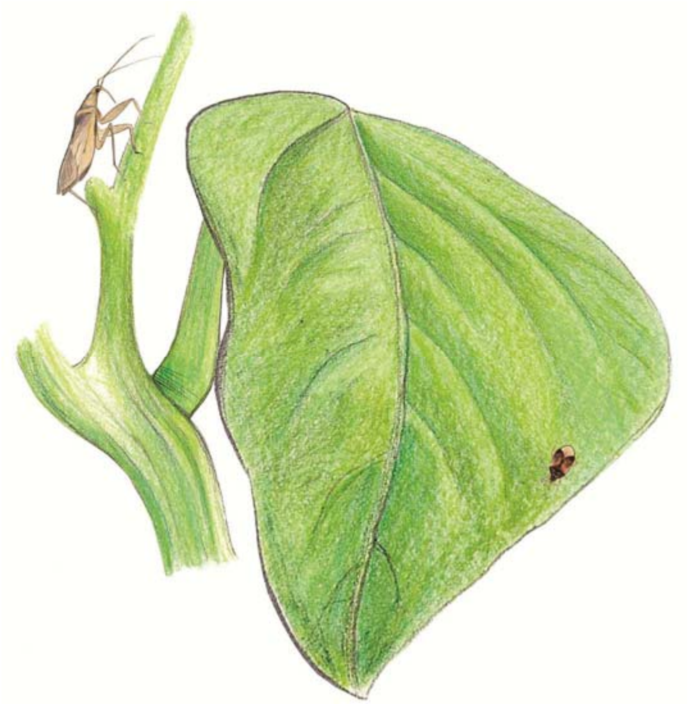
The influence of intraguild competitors on reproductive decisions by two predatory Heteroptera, *Orius insidiosus* (Anthocoridae) and *Nabis americoferus* (Nabidae)



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Front page: *Orius* spp. and *Nabis* spp. on a green bean stem

Resumen

El estudio de la relación entre los lugares en los cuales los depredadores realizan sus puestas y la depredación intragremial ha recibido poca atención hasta el momento, pero muy probablemente dicha depredación es un factor que condiciona la ecología reproductiva de muchos depredadores. En este trabajo se ha estudiado la intensidad de la oviposición y la distribución de las puestas por parte de *Orius insidiosus* (Hemiptera: Anthocoridae) y *Nabis americoferus* (Hemiptera: Nabidae) en plantas de judía tanto de manera individual como cuando las dos especies coexisten juntas. Ambos depredadores pusieron más huevos en presencia de la otra especie que cuando permanecieron sólo con individuos de su misma especie. En presencia de sólo conspecíficos, *O. insidiosus* puso más huevos en hojas y peciólulos en la mitad más alta de la planta mientras que *N. americoferus* puso la mayoría de los huevos en peciolos y peciólulos igualmente distribuidos a lo largo de toda la altura de la planta. Cuando las dos especies coexistieron, *O. insidiosus* prefirió poner sus huevos en las hojas mientras que *N. americoferus* alteró su comportamiento y puso una mayor proporción de sus huevos en peciolos y peciólulos. Ambos modificaron sus preferencias por diferentes estratos dentro de las plantas: *N. americoferus* puso más huevos en la parte superior de la planta cuándo la otra especie estaba presente y *O. insidiosus* puso marginalmente más huevos en zonas más bajas de la planta cuándo el otro depredador se encontraba presente. Este trabajo indica que estas dos especies de Cimicomorpha pueden detectar la presencia el uno del otro y que ambos ajustan en consecuencia sus decisiones reproductivas, presumiblemente para evitar posibles interacciones competitivas.

Abstract

The relationship between the oviposition site preferences of predators in the face of intraguild competitors has received little attention, but it likely shapes the reproductive ecology of predatory species. In this study, oviposition intensity and the within-plant distribution of *Orius insidiosus* (Hemiptera:Anthocoridae) and *Nabis americoferus* (Hemiptera: Nabidae) eggs on *Phaseolus vulgaris* plants was studied when the two species were present independently or in combination. Both predators laid more eggs in the presence of the other species relative to when they were only exposed to conspecifics. When only exposed to conspecifics, *O. insidiosus* preferred to lay eggs on leaves and petioles on the upper half of the plant, whereas *N. americoferus* laid eggs mostly on the petioles and petiolules equally throughout the height of the plant. But when both species were present, *O. insidiosus* preferred to lay eggs on the leaf whereas *N. americoferus* altered their behavior to lay an even greater proportion of their eggs on the petioles and petiolules. They altered their preferences for different plant strata too: *N. americoferus* laid more eggs on the upper quarter of the plant when *O. insidiosus* was present, and *O. insidiosus* was marginally more likely to lay eggs lower on the plant in the presence of *N. americoferus*. This study indicates that these two Cimicomorpha can detect the presence of one another, and that they adjust their reproductive decisions, presumably to avoid potential competitive interactions.

1. Introduction

Reproductive behavior of predatory arthropods influences the distribution and abundance of the predators within a habitat. Different factors influence the place where an egg is ultimately laid, ranging from the physiology of the mother and her egg, the biotic and abiotic characteristics of the environment, and the resource needs of the mother and her developing offspring (Seagraves, 2009; Lundgren, 2011). Their diverse reproductive behaviors and importance to biological control make predatory Heteroptera an excellent system to study how various constraints affect reproductive decisions.

In large part, the oviposition decision of predatory Heteroptera is affected by the relative survival and performance of the progeny. For predators that lay their eggs on plants, oviposition decisions are frequently influenced by plant characteristics and prey availability. Many cimicomorphan Heteroptera insert their eggs into plants at sites that promote offspring performance. For this reason, these species often have distinct preferences for different plants, and this is often manifested in increased offspring performance (Coll, 1996; Sanchez *et al.*, 2004; Lundgren and Fergen 2006; Lundgren *et al.*, 2008; Lundgren *et al.*, 2009). Within a plant species, these Heteroptera display additional preferences for specific plant tissues as oviposition substrates. Proposed mechanisms for these preferences include the thickness or resistance of the epidermis (Lundgren *et al.*, 2008; Seagraves *et al.*, 2011), the relative densities of trichomes or leaf textures (Benedict *et al.*, 1983; Richards and Schmidt, 1996b; Sigsgaard, 2004; Lundgren *et al.*, 2008), and the rate at which offspring are able to hatch from various structures (Groenteman *et al.*, 2006). Relative food availability (especially prey), may also affect the reproductive decision of a female. This is particularly well-studied in Anthocoridae. *Anthocoris nemorum* L., *A. nemoralis* Fabricius, and *A. confusus* (Reuter) all lay their eggs near high densities of prey (Evans, 1976; Sigsgaard, 2004, 2005). More recently, Seagraves and Lundgren (2010) demonstrated that plant quality was most important in influencing the oviposition behavior of *O. insidiosus*, but when plant identity was constant, this predator laid more eggs on the plants with high prey abundance. In predatory Heteroptera, one source of potential prey that has been poorly explored within the context of reproductive ecology is the presence of competitors' eggs.

Another factor that may influence the final oviposition decision of a female predator is the presence of other natural enemies and their interactions, as is the case of the intraguild predation. The ladybird *Coleomegilla maculata* (DeGeer) prefers to oviposit on plants with glandular trichomes (Griffin and Yeargan, 2002; Seagraves and Yeargan, 2006) or in areas far from large aphids aggregations (Schellhorn and Andow, 1999) to reduce predation on her eggs. Another predator that prefers to oviposit in sites with high trichome densities is the predatory midge *Aphidoletes aphidimyza* Rondani, whose females lay eggs in pubescent leaves where predation by coccinellids is reduced (Lucas and Brodeur, 1999). Two hoverfly species, *Episyrphus balteatus* De Geer and *Metasyrphus corollae* Fabricius (Diptera: Syrphidae), responded differently to the presence of ladybird larvae: *E. balteatus* females oviposited less in the presence of the coccinellids, whereas *M. corollae* behavior was largely unaffected (Putra *et al.*, 2009). Females of the predatory mite *Iphiseius degenerans* (Berl.) (Acari: Phytoseiidae) avoid predation on their eggs by ovipositing away from pollen resources, where their egg predators, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), forage for prey (Faraji *et al.*, 2001, 2002). There are some predators that even cease oviposition in the presence of other natural enemies: the predatory mite *Neoseiulus cucumeris* (Oudemans) is reluctant to oviposit in the presence of *I. degenerans* (Monserrat *et al.*, 2007).

Orius insidiosus is an important native predator of crop pests in many agronomic systems in North America. This species is widely collected throughout much of North America east of the Rocky Mountains (Shapiro *et al.*, 2010), and is often a dominant foliar-dwelling predator in major field cropping systems such as corn (Isenhour and Marston, 1981; Corey *et al.*, 1998; Seagraves and Yeargan, 2009), soybeans (Fox *et al.*, 2004; Rutledge and O'Neil, 2005) (Al-Deeb et al., 2001). The damsel bug *Nabis americoferus* Carayon is also a common generalist predator in Midwestern field crops (Braman and Yeargan, 1990) where it preys at least on leafminers (Guppy, 1986) and Lepidoptera (Sloderbeck and Yeargan, 1983). These two species of Heteroptera are sympatric in several crops, such as maize and beans (Coll and Bottrell, 1995)(Coll and Bottrell, 1995) or soybean (Isenhour and Yeargan, 1982; Clements and Yeargan, 1997). Within soybeans, *O. insidiosus* and *N. americoferus* prefer to reside on different plant parts, and are thus presumed to have minimal interactions (Clements and Yeargan, 1997). A recent literature review on the reproductive ecology of predaceous Heteroptera by Lundgren (2011) discusses the possibility of an avoidance behavior between predators, but few studies directly compare how predatory Heteroptera adjust their oviposition behavior to avoid intraguild interactions.

The objective of this study was to evaluate the interactions among these two sympatric species. Specifically we addressed the hypotheses that in the presence of heterogeneric competitors, these bugs alter their 1) oviposition intensity, and 2) their placement of eggs on a plant, relative to when these predators are only exposed to conspecifics.

2. Material and methods

Green bean plants (*Phaseolus vulgaris* L.) were grown in the greenhouse from seed in a compost/peat-moss/perlite mixture and watered daily. Plants used in the experiment were between 19 and 27 d old and had between two and six internodes. The medium height plant per treatment was as follows: 15.83 ± 1.45 (*N. americoferus*), 14.48 ± 1.34 (*O. insidiosus*) and 15.58±0.87 (both species together) with a total height per individual plant that varied from 5.3 to 25.9 cm.

Orius insidiosus and *N. americoferus* were field collected from alfalfa fields in Brookings, SD. Field-collected adults were bred and maintained in climatic chambers (25 ± 1ºC, 70 ± 10 % relative humidity and 16:8 light:dark [L:D]) for a maximum of two generations with *Ephestia kuehniella* Zeller (Lepidoptera:Pyralidae) eggs (Beneficial Insectary, Redding, CA), water provided with a moistened cotton wick and green bean pods or green bean plants for *O. insidiosus* and *N. americoferus*, respectively. Offspring from field-collected *O. insidiosus* and *N. americoferus* were used in the experiments.

The experimental unit consisted of a single potted green bean plant enclosed within a cylindrical cage (39 cm tall and 12.5 cm diameter with 0.33 mm square mesh). The soil was covered with white sand to facilitate searching for the insects. Two pieces ($1 cm^2$) of a Post-it® (≈$1 cm^2$; 3M St Paul, MN), each with approximately 550-600 eggs of *E. kuehniella* affixed were added per plant. These two pieces were placed in the middle part of the plant, depending on its height. Also a few thrips were provided as a food source. These plants were randomly assigned to one of three treatments: (1) four *O. insidiosus* females (n = 20 plants), (2) four *N. americoferus* females (n = 17) and (3) two *O. insidiosus* females together with two *N. americoferus* females. For the latter treatment, plants were only included in the analysis for a given species if they laid eggs; this resulted in a sample size of 14 and 12 for *O. insidiosus* and *N. americoferus*, respectively. The experimental conditions were 25 ± 1ºC, 70 ± 10 % relative humidity and 16:8 L:D and the insects were exposed to the plants for 48 h. After that time the plants were kept refrigerated until the eggs laid by each insect could be counted microscopically. The number of eggs laid was classified according to five different plant structures: floret, leaf, internode, petiole and petiolule (Lundgren and Fergen 2006). Also, the length of each plant was divided into four segments, and the number of eggs laid by each species in each stratum was recorded (from lowest to highest, the strata were designated I-IV).

A one-way ANOVA was used to determine differences in the total number of eggs laid per female and treatment, means were separated with the Tukey test ($P< 0.05$). Those values were log+1 transformed to ensure the homoscedasticity of variances. Two-way ANOVAs were used to compare the effects of our treatments on oviposition patterns of these two bugs, with treatment and plant structure or plant stratum as main factors in the analysis. Means were separated with the LSD test. Separate analyses were conducted on *N. americoferus* and *O. insidiosus*. The one-way analyses were conducted using SAS Enterprise Guide 4.2 while the two-way analyses were conducted using SYSTAT 11.

3. Results

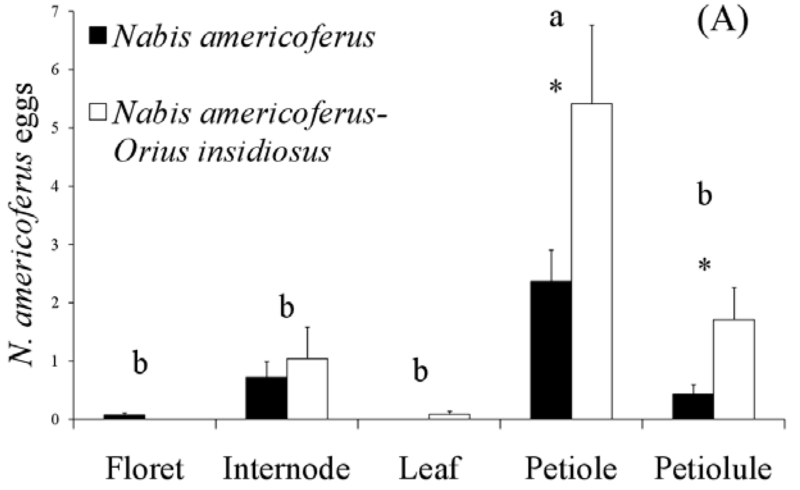
Nabis americoferus oviposition rate increased significantly and they preferred to lay eggs on different plant structures in the presence of *O. insidiosus* females than in the presence of conspecifics (treatment: $F_{1, 135} = 9.52$, $P = 0.002$; plant structure: $F_{4, 135} = 19.71$; $P < 0.001$; interaction: $F_{4, 135} = 3.67$, $P = 0.007$). *Nabis americoferus* laid significantly more eggs on petioles than anywhere else on the plants (Figure 4.1A), and eggs were laid at similar rates on the remainder of the plant structures. Petioles ($F_{1, 27} = 5.46$, $P = 0.03$) and petiolules ($F_{1, 27} = 6.41$, $P = 0.02$) were the only structures where eggs were significantly more abundant in the treatment with both bugs compared with the *N. americoferus*-only treatment; eggs were laid at similar rates in the two treatments on florets, internodes, and leaves.

In contrast, oviposition rates and preferences of *O. insidiosus* females on the different plant structures were unaffected by the presence of *N. americoferus* (treatment: $F_{1, 160} = 0.76$, $P = 0.38$; plant structure: $F_{4, 160} = 13.23$; $P < 0.001$; interaction: $F_{4, 160} = 1.23$, $P = 0.30$). However, even though differences were not significant ($F_{1, 32} = 0.17$, $P = 0.69$), *O. insidiosus* laid a slightly higher mean number of eggs per plant per female in the presence of *N. americoferus* (Table 4.1). *Orius insidiosus* laid the most eggs on leaves and petioles, and the least number of eggs on the florets and internodes (Figure 4.1B).

Table 4.1: Mean (SEM) number of eggs laid per female per plant on *Phaseolus vulgaris* by *Nabis americoferus* and *Orius insidiosus* when the bugs were caged alone and with the competitor. Sample sizes were 12 and 14 plants for *N. americoferus* and *O. insidiosus*, respectively. Strata are numbered lowest to highest; differences among strata (within a column) are indicated by lower case letters (columns without letters were not significant); asterisks indicate significant differences (α = 0.05) between columns within a species (differences between treatments within a stratum).

Stratum	*Nabis americoferus*		*Orius insidiosus*	
	Alone	With *Orius insidiosus*	Alone	With *Nabis americoferus*
I	0.46 ± 0.21	1.00 ± 1.00	0.04 ± 0.02^{b}	0.04 ± 0.03^{b}
II	1.16 ± 0.31	1.92 ± 1.13	0.71 ± 0.33^{b}	1.61 ± 0.58^{a}
III	1.12 ± 0.31	2.21 ± 0.81	1.89 ± 0.40^{a}	2.75 ± 0.49^{a}
IV	0.85 ± 0.29	3.00 ± 1.20*	2.40 ± 0.60^{a}	1.50 ± 0.36^{a}
Total eggs/ plant/ female	3.59±0.59	8.25±1.71*	5.04±0.51	5.89±0.92

The presence of *N. americoferus* did not affect the plant stratum where *O. insidiosus* preferred to lay eggs (treatment: $F_{1, 128} = 0.55$, $P = 0.46$; plant stratum: $F_{3, 128} = 12.23$, $P < 0.001$; interaction: $F_{3, 128} = 2.17$, $P = 0.09$). Overall, most *O. insidiosus* eggs were laid higher on the plant (strata III and IV) in both treatments. *Orius insidiosus* laid marginally more eggs lower on the plant when *N. americoferus* was present, and higher on the plants (stratum IV) in the absence of *N. americoferus* (Table 4.1). Although *N. americoferus* laid significantly more eggs when *O. insidiosus* females were present, they laid their eggs equally throughout the height of the plant regardless of the presence or absence of *O. insidiosus* (treatment: $F_{1, 108} = 5.91$, $P = 0.02$; plant stratum: $F_{3, 108} = 1.23$; $P = 0.30$; interaction: $F_{3, 108} = 0.58$, $P = 0.63$).



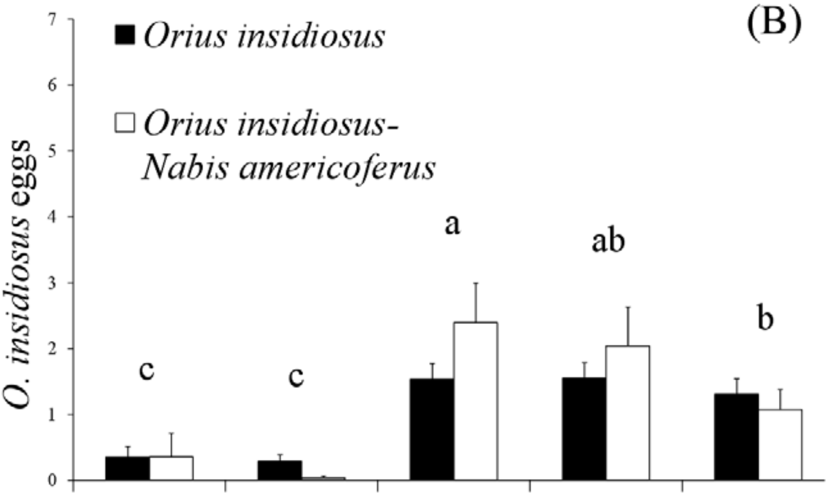


Figure 4.1: Mean (SEM) number of eggs laid per female per plant on different plant structures of *P. vulgaris* by *N. americoferus* (1A) and *O. insidiosus* (1B) alone and in the presence of their competitor.

4. Discussion

Orius insidiosus and *N. americoferus* are able to perceive the presence of interspecific competitors, and these predators adjust their oviposition patterns in response.

This research underscores that predators often prefer to lay eggs in certain strata throughout the height of a plant, and that the presence of interspecific competitors can alter these preferences (Table 2.1). In the absence of *N. americoferus*, *O. insidiosus* laid marginally more eggs in the upper strata (stratum IV) of the bean plants. In the presence of *N. americoferus*, *O. insidiosus* laid marginally more eggs in lower strata (strata II and III). Moreover, although it was not statistically significant, *N. americoferus* appeared to lay more eggs in lower strata (strata II and III) when *O. insidiosus* was absent; when *O. insidiosus* was present, *N. americoferus* laid more than three times more eggs high on the plant (stratum IV). The literature reveals that predatory heteropterans display oviposition preferences for areas along the height of a plant, but the literature suggests that these preferences are neither consistent among or with species. Van den Meiracker and Sabelis (1993) demonstrated that *O. insidiosus* prefers to oviposit in the growing tips of caged sweet pepper plants. Another study carried out by Graham and Jackson (1981) showed that *Orius tristicolor* (White) (Heteroptera: Anthocoridae) preferred to oviposit in the upper and longer stems of alfalfa plants. To the contrary *O. insidiosus* did not show clear preferences for ovipositing in specific nodes along the stem of green bean plants in a study carried out by Lundgren and Fergen (2006). The results obtained for *N. americoferus* in previous experiments depend on the plant species used. More eggs were found in the upper parts of alfalfa (Richards and Harper, 1978; Graham and Jackson, 1982) and soybean stems (Braman and Yeargan, 1989). Pfannenstiel and Yeargan (1998) found that eggs distribution appears to be dependent on plant morphological characteristics: *Nabis roseipennis* Reuter (Heteroptera: Nabidae) laid more eggs in the lower third of corn, tomato and tobacco but this same species laid more eggs in the middle and upper parts of squash and soybean. In a field experiment where *O. insidiosus* and *N. roseipennis* were presented together, *O. insidiosus* preferred to oviposit on the top portions of the plant and *N. roseipennis* restricted its oviposition to the mid-section of the plants (Isenhour and Yeargan 1982). Our results are consistent with the hypothesis that, in the other’s presence, *O. insidiosus* descends to lower levels and *N. americoferus* ascends to higher levels within a plant. It is also important to acknowledge that certain plant structures are found disproportionately across plant strata (for example, often more petioles are found higher on the plants), and thus the location of preferred plant structures could account for which plant strata are preferred.

In addition to plant stratum, the two predators studied showed distinct oviposition preferences for different plant structures in green bean plants (Figure 4.1). *Orius insidiosus* preferred to oviposit in the leaves and petioles, while *N. americoferus* preferred to oviposit in the petioles. Although they displayed preferences for different structures, avoidance was not complete; both species oviposited in petioles and petiolules. Moreover in the presence of *O. insidiosus*, *N. americoferus* laid significantly more eggs in the petioles and petiolules than in its absence. Seagraves et al. (2011) found that *O. insidiosus* preferred to oviposit in the leaves of green bean plants, but oviposition shifted to the petioles when these plants were water stressed. In contrast, Lundgren and Fergen (2006) found that most of the eggs laid by *O. insidiosus* in green bean plants were laid in the petiolules and petioles, and less in the leaves. Another anthocorid, *Anthocoris confusus*, laid the majority of its eggs in the leaves of broad bean plants in a long-term laboratory study, but this depended on the prey distribution on the leaves (Evans, 1976). In apple and pear leaves, *Anthocoris nemorum* preferred to lay their eggs near leaf margins on the ventral side of damaged leaves, whereas *Anthocoris nemoralis* laid their eggs on the leaf vein on the dorsal side of healthy leaves (Sisgaard 2004). In a laboratory experiment, Pfannenstiel and Yeargan (1998) found that *N. roseipennis* laid significantly more eggs in the petioles of soybean and squash plants than in any other plant structure. When eggs of both species were sampled at the same time in a soybean field, more *O. insidiosus* and *N. americoferus* eggs were found in the petioles while more *N. roseipennis* eggs were found in the petiolules (Isenhour and Yeargan, 1982). Clearly, the oviposition preferences for specific tissues by predatory heteropterans are quite plastic, influenced by the bug and plant species involved, and the current biotic (especially local resource availability) and abiotic conditions.

Finally, we hypothesize that avoidance of predation or competition is partially responsible for the shift in oviposition behavior by these predatory bugs. Some studies have observed predation on *O. insidiosus* in the field by other predator taxa: *N. americoferus* attacks *O. insidiosus* adults and nymphs in soybean fields (Braman and Yeargan, 1989), and *Geocoris* spp. attack *Orius* spp. in cotton fields (Loya-Ramirez *et al.*, 2003; Rosenheim, 2005). Thus, we suspect that eggs are at risk of predation by other predatory bugs, which may explain why eggs are inserted into plant tissue in the first place (Lundgren, 2011). Another observation we made was that *N. americoferus* *N. americoferus* increased oviposition 2.3-fold when *O. insidiosus* was present. There may be different explanations for this behavior. It is possible that preferred oviposition sites are limiting on a given plant, and reducing the number of conspecifics from four to two allowed the remaining females to exploit these preferred sites more completely. Groenteman et al. (2006) found that mated *O. albidipennis* females defended preferred oviposition sites along the veins of cotton leaves against other females. If *N. americoferus* perceives *O. insidiosus* as prey, the former may increase its ovipositon rate to fully exploit this food source. Finally, plants defend themselves from traumatic oviposition by predatory Heteroptera (De Puysseleyr *et al.*, 2011), and the alterations in the plant's physiology could affect subsequent oviposition behavior of predatory bugs. Differences in oviposition intensity when interspecific competitors were present or absent was greater for *N. americoferus* than for *O. insidiosus*. It is possible that these two bugs affect this immune response of the plant differently and that this might explain this pattern. We conclude that oviposition preferences of important predators are influenced by intraguild interactions and to fully understand the mechanisms involved in this behavior, as well as its implications under more realistic conditions, additional future work is warranted.

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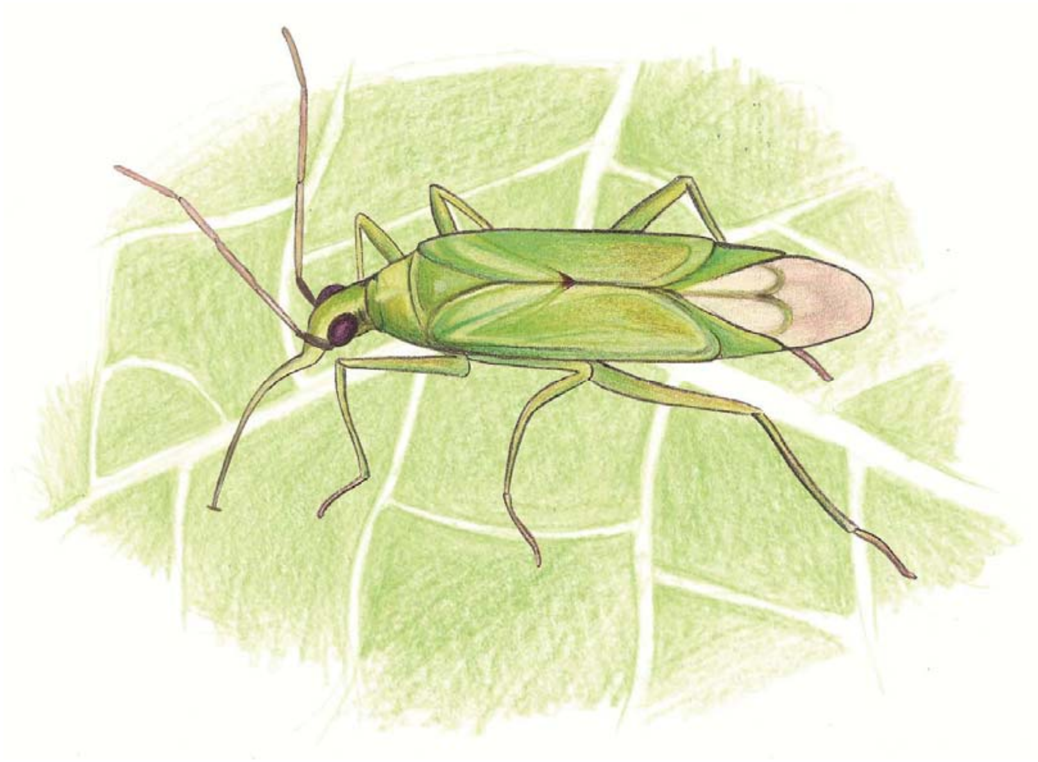
Development of specific ITS markers for DNA identification within herbivorous insects



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Front page: *Macrolophus* spp. feeding on a tomato leave

Resumen

Las técnicas basadas en la detección de ADN han demostrado ser métodos muy útiles para el estudio de las relaciones tróficas entre las plagas y sus enemigos naturales. Ahora bien, ya que la mayoría de los depredadores son omnívoros, la identificación de ADN vegetal permitiría la identificación de la planta en la cual el depredador se ha alimentado previamente. En este trabajo se ha desarrollado la técnica PCR mediante el diseño de marcadores específicos de planta como método para detectar ADN vegetal dentro del aparato digestivo de un depredador omnívoro (*Macrolophus pygmaeus*) y dos especies plaga (*Helicoverpa armigera* y *Tuta absoluta*). Para ello se diseñaron marcadores específicos de tomate de las regiones ITS 1 y 2 que permitieron la amplificación de un fragmento de ADN de tomate de 332 pb en el interior de las tres especies en todos los casos (100% de detección a t=0) así como no amplificaron ADN ni de otras especies vegetales ni de los insectos en ayunas. El 50% de detección del ADN de tomate a 25ºC varió entre 5.8, 27.7 y 28.7 horas en *M. pygmaeus*, *H. armigera* y *T. absoluta*, respectivamente. A su vez con estos marcadores se identificó ADN de tomate en *M. pygmaeus* recolectados en campo, lo cual sugiere la existencia de una dieta mixta en este depredador omnívoro, que fue mayor en hembras y ninfas que en machos. Este estudio proporciona una herramienta útil para detectar e identificar fuentes vegetales de alimento de los artrópodos y para evaluar la colonización de los cultivos desde la vegetación circundante en programas de control biológico por conservación.

Abstract

DNA-based techniques have proved to be very useful methods to study trophic relationships between pests and their natural enemies. However, most predators are best defined as omnivores, and the identification of plant-specific DNA should also allow the identification of the plant species the predators have been feeding on. In this study, a PCR approach based on the development of specific primers was developed as a self-marking technique to detect plant DNA within the gut of one heteropteran omnivorous predator (*Macrolophus pygmaeus*) and two lepidopteran pest species (*Helicoverpa armigera* and *Tuta absoluta*). Specific tomato primers were designed from the ITS 1–2 region, which allowed the amplification of a tomato DNA fragment of 332 bp within the three insect species tested in all cases (100% of detection at t=0) and did not detect DNA of other plants nor of the starved insects. Plant DNA half-lives at 25°C ranged from 5.8 h, to 27.7 h and 28.7 h within *M. pygmaeus*, *H. armigera* and *T. absoluta*, respectively. Tomato DNA detection within field-collected *M. pygmaeus* suggests dietary mixing in this omnivorous predator and showed a higher detection of tomato DNA in females and nymphs than males. This study provides a useful tool to detect and to identify plant food sources of arthropods and to evaluate crop colonization from surrounding vegetation in conservation biological control programs.

1. Introduction

In nature, predators and parasitoids move over a broad range of spatial scales, and it is of vital importance to understand the dispersal characteristics of these beneficial insects for biological control of agricultural pests. Different marking and tracking techniques have been developed in order to evaluate the movement or identify the sources of several species of arthropods (Lavandero *et al.*, 2004; Jones *et al.*, 2006; Wanner *et al.*, 2006; Goubault and Hardy, 2007; Scarratt *et al.*, 2008; Stephens *et al.*, 2008). However, those useful methods have a major inconvenience for field studies; either the insects or the plants need to be marked beforehand.

Many predators are omnivores, consuming plant provided foods at least during part of their life cycles (Albajes and Alomar, 2004; Wäckers, 2005). Enhancing the availability of such food sources within or close to crops provides resources, which enhance populations of natural enemies, and adds to their colonization of the target crop, increasing the effectiveness of biological control (Landis *et al.*, 2000; Gurr *et al.*, 2004). Pollen grains, present either on the exoskeleton or within the gut, have been used to confirm feeding on certain plant species (Silberbauer *et al.*, 2004). However, procedures for morphological pollen identification are too time consuming, and not all predators feed on pollen.

In recent years, several studies have developed DNA-based techniques to analyse predator gut contents in arthropods, mainly in those where the feeding does not leave remains that can be morphologically identified. The first attempts developed specific SCAR (sequence characterized amplified region) markers (Agustí *et al.*, 1999, 2000); but, more recently, other regions like the internal transcribed spacer region 1 (ITS-1) (Hoogendoorn and Heimpel, 2001) or the cytochrome c oxidase subunits I and II (COI and COII) mitochondrial genes have been used to develop prey-specific primers (Agusti *et al.*, 2003; Greenstone *et al.*, 2007; Weber and Lundgren, 2009). Based on this, an alternative way to track movement of omnivorous predators from their refuges would be the identification of ingested plant DNA within whole insects, as similarly done in predation gut contents analysis studies. Even if the COI region has been mainly used for primer design in gut analysis of predation, it is not clear which region would be most appropriate for the detection of ingested plant DNA. ITS 1–2 together with *trnH-psbA* region have been proposed to have a faster gene evolution rate than COI in plants (Chase *et al.*, 2005).

Macrolophus pygmaeus (Rambur) (Heteroptera: Miridae) is a polyphagous predator that feeds on several arthropod species. Until recently, *M. pygmaeus* on tomato has been misidentified as *M. melanotoma* (Costa) (=*M. caliginosus* Wagner) and is still named as *M. caliginosus* by commercial beneficial producers (Martinez-Cascales *et al.*, 2006). This species spontaneously colonizes field and greenhouse crops from refuges present in the agricultural landscape of the Mediterranean basin (Alomar *et al.*, 2002; Gabarra *et al.*, 2004; Castane *et al.*, 2004). Like most mirids, it is an omnivore that also feeds on plant tissues; therefore, it was selected as a candidate for our study. Because *M. pygmaeus* is a small sucking insect and it is not known whether it feeds either on phloem or on leaf cells, we suspected that prohibitively small quantities of plant DNA would be present in its gut leading on a low detection of plant DNA. For this reason, we also tested two insects with chewing habits that would ingest a large amount of plant cells: *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae, both important tomato pests.

Here, we show that molecular markers can be used to specifically identify plant DNA in herbivorous/omnivorous insects. We have developed a tomato-specific marker which allows the detection of tomato DNA in the gut of three different insect species with different feeding types (sucking or chewing insects) and showed the detection percentages of tomato DNA within their gut with digestion time. Finally, we have also shown that this marker allows the identification of plant DNA within field insects collected in tomato greenhouses with just a PCR reaction, avoiding the process of sequencing.

2. Material and methods

2.1 Insects and plants

Macrolophus pygmaeus were reared at IRTA facilities as explained by Agustí and Gabarra (Agustí and Gabarra, 2009). This colony is renewed every year with introductions of new field collected insects near Barcelona (NE Spain). They were maintained on tobacco plants (*Nicotiana tabacum* L.) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. *Helicoverpa armigera* and *T. absoluta* larvae were collected in tomato fields near Barcelona and maintained on artificial diet and on tomato plants, respectively. All insects were maintained under controlled conditions of 25±1°C, 70±10% RH and L16:D8 photoperiod. *Ephestia kuehniella* eggs were provided by Biotop (Valbonne, France).

Tomato (*Solanum lycopersicum* L.), cabbage (*Brassica oleracea* L.) and tobacco (*Nicotiana tabacum* L.) plants were cultivated in greenhouses at IRTA. Potato (*Solanum tuberosum* L.), aubergine (*Solanum melongena* L.), pepper (*Capsicum annum* L.), zucchini

(*Cucurbita pepo* L.) and cucumber (*Cucumis sativus* L.) plants were obtained from fields in the vicinity of our facilities. *Carlina corymbosa* L., *Ononis natrix* L., *Verbascum thapsus* L. and *Solanum nigrum* L. plants were obtained from the margins of the previously cited crops in the same area.

2.2 DNA extraction

Whole individual insects were homogenized in clean microcentrifuge tubes to avoid possible contamination by its own faeces, and DNA extractions were done using the DNeasy Tissue Kit (QIAGEN, Hilden, Germany; protocol for insects). Plant DNA was extracted from a 1 cm diameter leaf disc using the DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's protocol. Total DNA was eluted with 100 ml in the AE buffer provided in the kit. All DNA extracts were stored at –20°C.

2.3 PCR amplification

Specific tomato primers were designed from the ITS 1–2 region by comparison with sequences of other solanaceous plants with CLUSTALW (Larkin *et al.*, 2007). Sequences obtained from GenBank were: *S. lycopersicum* (AF244747), *S. tuberosum* (AY875827), *S. nigrum* (AJ300211) and *N. tabacum* (AJ300215). Guidelines proposed for the design of efficient and specific primers by Innis & Gelfand (1990) and Saiki (1990) were followed. Primers were synthesized by Roche Diagnostics, Basel, Switzerland. DNA amplifications were performed in a 10 µl reaction volume containing 1 µl of DNA extract, 5 µl of master mix of Multiplex Kit (QIAGEN) and 1 µl of primer mix (10 µM). Samples were amplified in a 2720 thermal cycler (Applied Biosystems, CA, USA) for 40 cycles at 94°C for 30 s; 62°C for 2 min and 72°C for 90 s. A first cycle of denaturation at 95°C for 15 min and a final extension at 72°C for 10 min were carried out. Tomato DNA and water were always included as positive and negative controls, respectively. PCR products were separated by electrophoresis in 1.5% agarose gels stained with ethidium bromide and visualized under UV light.

2.4 Species specificity

The specificity of the tomato primers was tested by attempting to PCR-amplify DNA from leaf discs of 11 other cultivated and non-cultivated plant species belonging to six families (Table 5.1) (*n*=2). These species were all selected as being present in the studied area and could potentially be fed on by the targeted insects. Starved *M. pygmaeus*, *H. armigera* and *T. absoluta* (*n*=10) were also tested.

Table 5.1: Plant species used in the specificity test (*n*=2)

Family	Species
Solanaceae	*Solanum lycopersicum* L., cv Bodar *Solanum tuberosum* L., cv. Red Pontiac *Solanum melongena* L., cv Cristal *Capsicum annum* L.,cv. Aristocrata *Nicotiana tabacum* L., cv. Brazillan Blend *Solanum nigrum* L.*
Cucurbitaceae	*Cucumis sativus* L., cv. Porto *Cucurbita pepo* L., cv. Mastil
Cruciferae	*Brassica oleracea* L., cv. Savoy
Compositae	*Carlina corymbosa* L.*
Papilionaceae	*Ononis natrix* L.*
Scrophulariaceae	*Verbascum thapsus* L.*

2.5 Feeding trials and detection periods

Clean tomato leaves were cut in small discs (2.5 cm diameter) which included a fragment of the central leaf vein, where mirids usually feed. Each tomato leaf disc was put on a 0.5-cm thick layer of an agar solution (5‰) in small plastic boxes (2.6 cm diameter). A starved 48 h (at 25°C) *M. pygmaeus* female was introduced in each plastic box for three hours at room temperature and was observed every 10 min. Only those individuals that had been seen with the stylet inserted into the leaf at least three times were considered to have fed and were frozen until tested by PCR. Lepidopteran feeding trials were prepared in the same way, but with a tomato leaf disc of 1 cm diameter and without the agar layer. In each of the plastic boxes, a third or fourth instar larva starved for 48 h was individually confined for three hours at room temperature. Lepidopteran larvae were only considered for the analysis if they had consumed at least 10 mm^2 of the tomato leaf.

Once the insects had been observed feeding, they were either immediately frozen at –20°C (t=0) or maintained at 25°C for 2, 4 or 8 h (*M. pygmaeus*); 6, 8 or 24 h (*H. armigera*) and 8 or 24 h (*T. absoluta*). After that, they were frozen at –20°C. Twenty *M. pygmaeus* females were assayed at t=0 and 8 h and 16 at t=4 h. Ten lepidopteran larvae were assayed for all times. Positive (tomato DNA) and negative (free DNA) control samples were included in all PCRs. Each sample was tested up to three times and considered positive if tomato DNA was detected in one of these three replicates. Negative exponential equations were fitted to describe the decay in the percentage of positive responses with time and R^2 was calculated (JMP 8.0.1; SAS Institute Inc.). From these equations, the half-lives (50% positive detection) were estimated (Greenstone *et al.*, 2007).

2.6 Analysis of field collected *Macrolophus pygmaeus*

We analyzed *M. pygmaeus* collected from several tomato greenhouses in the studied area. Those predators were part of another study that has analyzed predation on two whitefly species and their parasitoids (Moreno-Ripoll *et al.*, 2009, 2010). Once collected, those predators (25 males, 31 females and 83 nymphs) were frozen at –20°C prior to DNA extraction. Each individual was analyzed by PCR using the tomato specific primers developed in this work.

3. Results

3.1 Development of ITS markers

Sequences of ITS-1, 5.8S and ITS-2 regions of *S. lycopersicum*, *S. tuberosum*, *S. nigrum* and *N. tabacum* were aligned and compared in order to design one pair of tomato-specific primers. Their sequence was 5’-CCGAGGCGCGCAAGCTCTTC-3’ and 5’-TAAAGCCTTGCGGCGTGCGAG-3’ and the primers were named as Le2F and Le1R, respectively. They amplified a fragment of 332 bp for *S. lycopersicum* including part of ITS-1 and ITS-2, and the whole 5.8S region.

3.2 Species specificity and detection periods

These primers were tomato specific. No other plant species, neither cultivated nor weeds, amplified a band of the same length in the cross-reactivity test (Figure 5.1). Even if a band of a very high molecular weight was amplified in *N. tabacum*, this does not interfere with detection of the specific tomato band.

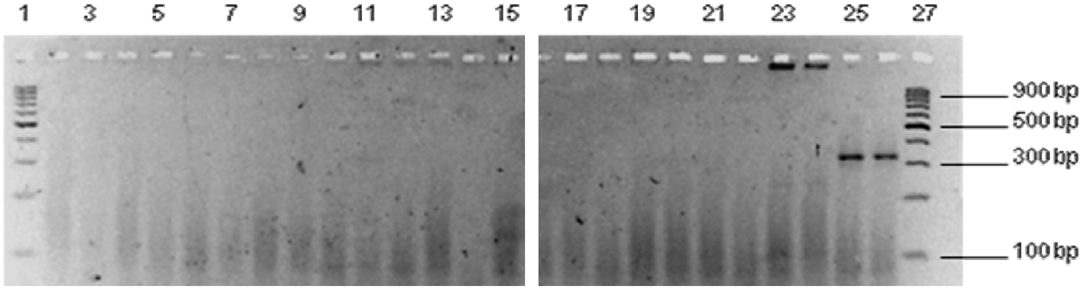

Figure 5.1: PCR products obtained using the tomato-specific ITS primers (332 bp). Lanes 3–26 show different plant species: 3–4, *Verbascum Thapsus*; 5–6, *Ononis natrix*; 7–8, *Carlina corymbosa*; 9–10, *Brassica oleracea*; 11–12, *Cucurbita pepo*; 13–14, *Cucumis sativus*; 15–16, *Capsicum anuun*; 17–18, *Solanum nigrum*; 19–20, *Solanum melongena*; 21–22, *Solanum tuberosum*; 23–24, *Nicotiana tabacum*; 25–26, *Solanum lycopersicum*. Lane 2, negative control. Lane 1 and 27, 100 bp molecular-size marker.

Besides, a fragment of that size is unlikely to be detected after digestion. None of the starved insects tested (*M. pygmaeus*, *H. armigera* and *T. absoluta*) gave false positive results (Figure 5.2).

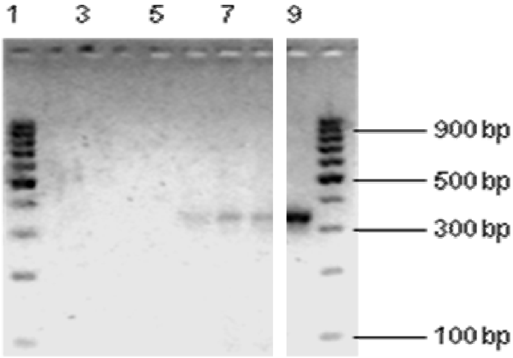

Figure 5.2: PCR products obtained using the tomato-specific ITS primers (332 bp). Lane 3, starved *M. pygmaeus*; lane 4, starved *T. absoluta*; lane 5, starved *H. armigera*; lane 6, *M. pygmaeus* fed on tomato; lane 7, *T. absoluta* fed on tomato; lane 8, *H. armigera* fed on tomato; lane 9, tomato. Lane 2, negative control. Lane 1 and 10, 100 bp molecular-size marker.

Tomato DNA was detected within all individuals of the three insect species tested after they had fed on tomato leaf discs with 100% detection in both chewing and sucking insects immediately after feeding (t=0) (Figure 5.2). In all three species, tomato DNA detection decreased with time since t=0 within *T. absoluta* and after 2 h and 6 h within *M. pygmaeus* and *H. armigera*, respectively (Figure 5.3). Detection curves were fitted to a negative exponential equation, starting with the last detection time where 100% detection was obtained. Equations were: $y=133.1\exp^{-0.17x}$, $R^2=0.98$; $y=114.7\exp^{-0.03x}$, $R^2=0.95$ and $y=88.7\exp^{-0.026x}$, $R^2=0.81$ for *M. pygmaeus*, *H. armigera* and *T. absoluta*, respectively. From these equations, half-lives of tomato DNA detection within their gut were estimated at 5.8 h for *M. pygmaeus*, 27.7 h for *H. armigera* and 28.7 h for *T. absoluta*.

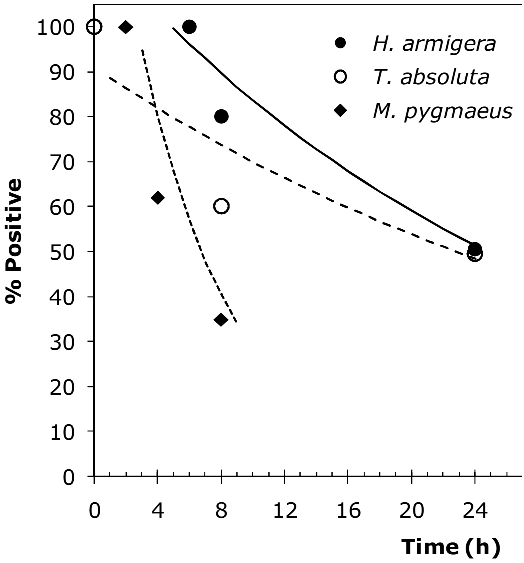

Figure 5.3: Detectability of tomato DNA in the gut of *M. pygmaeus, T. absoluta* and *H. armigera* at different times after ingestion. Equations and R^2 values are shown in the text.

3.3 Analysis of field collected *Macrolophus pygmaeus*

Tomato DNA was found in 30.2% of field-collected *M. pygmaeus* (*n*=139), being much higher in nymphs (36.1%) and females (32.3%) than in males (8%).

4. Discussion

In this study, we show the detection of plant DNA within the gut of three insect species by the use of a specific molecular marker. Tomato-specific primers were highly specific, showing no cross-reactivity either with other closely-related plant species or with the insect species tested.

The COI region has been applied extensively in animal barcoding; but, it is known that, for most of the plant species, it is not suitable due to its much slower rate of COI gene evolution in higher plants than in animals (Kress *et al.*, 2005). There is a lack of consensus on the most appropriate barcoding locus and criteria to be used in plants (Hollingsworth *et al.*, 2009; Valentini *et al.*, 2009). Kress *et al.* (2005) proposed ITS and *trnH-psbA* as the best candidate regions for the design of plant-specific molecular markers, and ITS has been shown to work on many plant groups and has been recommended (Chase *et al.*, 2005; Sass *et al.*, 2007; Chen *et al.*, 2010). According to these considerations, we have designed a pair of primers from the ITS region that amplifies a fragment,332 bp long, that proved very effective for the detection of tomato DNA within the gut of the insects tested. As previously suggested (Agustí *et al.,* 1999), those primers were designed to amplify relatively short fragments to make possible the detection of semi-digested DNA fragments.

In this study, tomato DNA was detected in both a small sucking insect (around 4 mm long) (*M. pygmaeus*) and two bigger chewing insects (*T. absoluta* and *H. armigera*). Even with this sucking insect, where the amount of ingested DNA was expected to be much lower than the bigger amount of leaf material (and then plant DNA) ingested by the chewers, the detection was possible in 100% of cases at t=0. As we expected, a faster loss of detection was found within the sucking insect. Some other authors obtained longer detection periods within other sucking insects species compared with chewing ones (Greenstone *et al.*, 2007; Hosseini *et al.*, 2008); but, as they also mention, detection depends not only on the size of the species analyzed but on the species itself. Degradation of the plant DNA through digestion probably also depends on other biotic and abiotic factors, as happens with insect DNA (Lövei *et al.*, 1990; Agusti *et al.*, 1999; Weber and Lundgren, 2009).

Tomato DNA was identified in many field individuals of unknown age and feeding history, which shows that even with a relatively quick digestion of tomato DNA within *M. pygmaeus*, this technique is useful to identify plant DNA in the gut contents of field-collected insects. As with other predators (Agustí *et al.*, 2003; Harwood *et al.*, 2007; Juen and Traugott, 2007), it is possible to analyze feeding events in the field and opens the possibility for more detailed studies to confirm the use of a range of food plants.

Such techniques can also be used to understand trophic interactions of omnivorous predators. In predatory Heteroptera, the functions of omnivory and the functional relationships between plant and prey feeding are still poorly understood; and it is not clear to what extent they depend on relative availability, amount or nutritional value of the food types (Gillespie and McGregor, 2000; Sinia *et al.*, 2004; Albajes *et al.*, 2006). In some cases, the digestive capabilities of these omnivorous predators may vary through their lives (Lundgren and Weber, 2010). Comparing our data with those obtained by Moreno-Ripoll *et al.* (2009) using specific primers of two whiteflies and their parasitoids, 13.7% of all individuals were positive for both tomato and insect prey, whereas only-plant or only-prey remains were found in 16.6% and 25.9% of their guts. Simultaneous detection of both food sources was much higher on nymphs than on females and males (19.3%, 6.5% and 4%, respectively). The fact that tomato DNA was detected in many field-collected *M. pygmaeus* clearly shows that plant material was consumed within a few hours of capture. Detection of both plant and prey within the same individual suggests dietary mixing, mainly in nymphs, according to a model where plant feeding is essential for predation (Sinia *et al.*, 2004).

Recently, some studies (Miller *et al.*, 2006; Matheson *et al.*, 2007; Jurado-Rivera *et al.*, 2009; Valentini *et al.*, 2009b) have identified plant meal composition in insects by molecular methods. In these studies, plant DNA fragments from insect guts were sequenced and compared for homologies in the BLAST database (http://blast.ncbi.nlm.nih.gov) in an attempt to identify the ingested plant species. Such procedures, a very powerful tool when identifying unknown ingested plants, are not very practical in field studies where the aim is to confirm the ingestion of a limited number of host plants and where a very high number of insects should be analyzed (e.g. to confirm plant sources of predators in crop colonization studies). That would not only require sequencing each DNA fragment found in their gut, but even cloning each fragment when several DNA fragments are present within the insect at the same time. In this case, it is much cheaper and more suitable to develop specific plant primers, in order to identify plant DNA with just a PCR as it has been done in most of the studies about molecular detection of predation and parasitism (Agusti *et al.*, 2005; Gariepy *et al.*, 2007; King *et al.*, 2008). If several plant DNAs are expected, a multiplex PCR can be used by developing one specific pair of primers for each of the plant species, avoiding the cloning and sequencing needed in the previously cited studies.

This study shows the detection of tomato DNA within the gut of insects by using a specific molecular marker. This marker allows knowing the percentage of insects which have been found to consume tomato plant in an insect population. This is a promising technique in conservation biological control because it can speed up the identification of food plants of colonizing species in the agricultural landscape surrounding target crops.

Acknowledgements

We thank Rafael Moreno-Ripoll and Rosa Gabarra for sharing DNA extractions of field-collected *M. pygmaeus* in order to analyze tomato DNA gut contents. We also thank Thaïs Aznar for her technical assistance and two anonymous reviewers for their comments. This work was funded by projects AGL2006-08726 and AGL2008-00546. NA was supported by the Ramon y Cajal Program and LP by a FPI doctorate studentship both from the Spanish Ministry of Science and Innovation (MICINN).

Enhancement of vineyard ecosystem services by under vine mulches

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Front page: Mulches on a vineyard

Resumen

Este trabajo investiga el papel de una serie de mantillos orgánicos en las viñas de dos viñedos en Nueva Zelanda durante un periodo de quince meses en cuanto a la mejora del micro clima en él árbol, las poblaciones de artrópodos epígeos, las poblaciones plaga, así como la calidad de la uva y la cosecha. Los mantillos usados en uno de los viñedos fueron paja de guisante, paja de lino, hierba segada así como un control en el que se dejó solo el suelo sin ninguna intervención; en el otro viñedo se usaron los mismos tratamientos con la excepción de la paja de guisante. En general, se encontraron más artrópodos en los mantillos comparado con el tratamiento control en ambos viñedos. Los mantillos redujeron las fluctuaciones en la temperatura del suelo así como aumentaron la humedad del mismo y los números de lombrices de tierra. No hubo efecto de los mantillos en la depredación de huevos de la plaga *Epiphyas postvittana* en la parte aérea en ninguno de los dos viñedos. Sin embargo los números de larvas de dicha plaga así como los daños asociados a ella durante la cosecha fueron menores en los tratamientos en los que se habían usado mantillos. La incidencia de botrytis durante la cosecha a su vez también fue menor en aquellos tratamientos donde se usaron los mantillos. El número de frutos pequeños o rotos fue menor en las viñas en las que se aplicó la paja de lino; en éste mismo tratamiento la cosecha fue mayor aunque la concentración de azúcar de la uva fue algo menor. En general este trabajo demuestra que un uso apropiado de estos mantillos orgánicos en los viñedos puede mejorar las condiciones del sistema así como proporcionar una serie de servicios ecosistémicos y mejorar la calidad de la uva y la cosecha. Los resultados obtenidos variaron en función del tipo de mantillo usado y del viñedo, pero el potencial de esta técnica merece una mayor profundización y estudio.

Abstract

This work investigated the role of a range of organic mulches under vines in two contrasting New Zealand vineyards over fifteen months, in enhancing vine micro-climate, epigeal arthropod populations, pest populations and grape yield and quality. Mulches at one site were pea straw, linseed straw and grass clippings, with a bare-ground control; at the second site, no pea straw was used. Overall, more arthropods were found on the mulches compared with the bare soil at both vineyards. Mulches reduced soil temperature fluctuations and increased soil moisture and earthworm populations. There was no effect of the mulches on light brown apple moth eggs predation rate in the canopy at either vineyard. However, light brown apple moth larval populations and associated damage during harvest were lower where mulches had been used, as was botrytis incidence. Fewer berries were under-sized or split when linseed straw was deployed. Yield was highest with linseed straw although berries sugar concentration was somewhat lower. Overall, the work showed that appropriate deployment of organic waste materials derived from within or outside vineyards ameliorated environmental extremes and enhanced a range of ecosystem services, as well as improving grape quality and yield. These results varied with vineyard location and mulch type but the potential of appropriate mulch use below vines merits further exploration.

1. Introduction

Viticulture worldwide is usually based on conventional, monocultural growing practices including the use of artificial fertilizers, pesticides and herbicides. As a result, ecosystem services in vineyards are usually at a low level because artificial inputs have taken their place. There is the potential for New Zealand and other countries to market wine as an environmentally sustainable product with low or zero chemical residues. New Zealand and other consumers have a strong preference for wine produced using sustainable production practices .

The retail sector has responded to consumers' demands for sustainably and ethically produced food, especially through large supermarket chains and the rise of local farmers markets (Codron et al., 2006). Environmental issues can be used by governments to adopt non-tariff trade regulations restricting market access for producers. The safety of vineyard workers who regularly apply pesticides is also of concern to consumers (Baldi et al., 2006). As a consequence, grape growers are seeking more sustainable growing practices with the aim of reducing agrochemical use in vineyards and enhancing ecosystem services (Fiedler et al., 2008).

Growers have adopted strategies that include conservation biological control and other ecological engineering initiatives that increase shelter and food resources for beneficial arthropods (Landis *et al.*, 2000; Gurr *et al.*, 2004; Sandhu *et al.*, 2008). The use of annual cover crops between rows in vineyards provides a source of nectar and pollen for parasitoid wasps and other natural enemies. Berndt et al. (2006) showed that the use of buckwheat (*Fagopyrum esculentum* Moench) can increase the longevity and fecundity of *Dolichogenidea tasmanica* Cameron (Hymenoptera: Braconidae), a parasitoid of the leafroller *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae) and reduce leafroller populations.

In relation to vine diseases, Jacometti (2007a, b) studied the effect of mulching on the biological control of botrytis disease, caused by *Botrytis cinerea* (Pers.: Fr) in vineyards. They found that mulching with shredded paper and composted grape marc significantly reduced the number of infected grape berries in the vineyard compared with unmulched plots. Mulching led to a reduction in *B. cinerea* sporulation and this was significantly correlated with increased rates of debris decomposition and increased soil biological activity. They also found that mulching led to elevated calcium concentrations in the soil which increased grape skin thickness, making grapes more resistant to *B. cinerea* infection.

Organic mulches can provide multiple ecosystem services such as weed control (Elmore *et al.*, 1997; Brown and Tworkoski, 2004), improved soil fertility (Agnew et al., 2005) and soil water infiltration (Buckerfield and Webster, 2001), reduced soil temperature fluctuations (Mundy and Agnew, 2002) as well as enhanced soil formation by earthworms (Buckerfield and Webster, 1996; Barre *et al.*, 2009).

However, little research has assessed the impact of mulches in vineyards on soil biodiversity and its contribution to ecosystem services other than earthworms. Thompson and Hoffmann (2007) studied the effect of under-vine mulching on the abundance of pests, their natural enemies and soil macro invertebrates in a vineyard in Victoria, Australia. They found that mulching increased the numbers of ground beetles, parasitoid Hymenoptera, spiders and earthworms. However, that work used pitfall traps, which are not designed for non-epigeal arthropods, such as most Hymenoptera. Also, the work did not attribute ecosystem functions to the arthropod taxa collected. Jacometti (2007a, b) showed that plant disease incidence is reduced by deployment of some vine mulches.

The present study considers the value of under-vine mulching in two New Zealand vineyards for increasing the biodiversity of soil invertebrates and the enhancement of ecosystem functions in the soil. First, we compare the abundance and assemblages of macrofauna associated with three different organic mulches. Secondly, we compare invertebrate predation rates in the vines' canopy, in relation to the under-vine treatment. Then, we assess the role that mulches may have on soil attributes such as temperature variation, water infiltration, microbial activity, soil nutrition and moisture retention. Finally, we evaluate the effect of these mulches on key aspects of grape yield and quality.

2. Materials and Methods

2.1 Study areas and experimental design

The field experiments were conducted in two vineyards located in Canterbury province, New Zealand, from February 2009 to May 2010. One, a conventionally managed site, was located in Waipara (43°04'42 S, 172°45'03 E, 62 m above sea level) and the other, a field characterized by the low inputs received, at Tai Tapu (43°42'25 S, 172°34'17 E, 23 m above sea level). The Waipara vineyard (Mud House Winery and Cafe) study site covered 0.54 ha of 5 year old Pinot Gris vines orientated north-south. The Tai Tapu vineyard (Bentwood Winery) site covered 0.7 ha of 17 year old Pinot Blanc vines orientated east-west. Both vineyards were irrigated by under-vine drippers. No insecticides were applied in either vineyard during this research. At Waipara, weeds were managed under-vines in this experiment with glyphosate (Polaris^{TM}) at 3 L/ha (monthly between August and December) and oxyflurofen (Goal^{TM}) at 3.75 L/ha (one application in August). The herbicides were applied only over the bare–ground control plots. Fungicides used were organic JMS Stylet oil^{TM} 12 L/ha (two applications in September) and sulphur (Kumulus^{TM}) at 8 kg/ha (monthly between October and December). The soil received annual dressings of fine lime at 200kg/ha and reactive phosphate rock (RPR) as 15S^{TM} at 150 kg/ha. The soil type at the Waipara vineyard was silt loam with a pH of 6.5. Prunings were mulched into the soil every winter. Ryegrass (*Lolium perenne* L.) was sown in the inter-row space of every second row and mown monthly. Buckwheat was sown in every fifth inter-row. This provided a nectar resource for parasitoids of key herbivore pests and was subsequently planted every 6 weeks to provide a continual nectar supply for these key parasitoids (Nafziger and Fadamiro, 2011). At Tai Tapu on September 19 and December 12, 2009, glyphosate G360 (1% v/v) and paraquat-diquat Brown Out (0.125% v/v) were applied at the rate of 3 L/ha. The fungicide used was sulphur at 5 kg/ha (one application in February, three in October and two in November). The organic fertilizer Ectol was applied twice in October and November at 3 L/ha. The soil type at the Tai Tapu vineyard was clay loam with a pH of 5.6. Strips of ryegrass were grown in the inter-row space and were mown on a monthly basis.

In Waipara, four different experimental treatments were established: bare soil (control), pea straw, linseed straw and grass clippings. In Tai Tapu only three treatments were used: the same as above, except for pea straw. All the treatments were applied directly under the vines between the end of February and the beginning of March 2009. The straw mulches and the grass clippings were spread around every vine in a rectangle of 150 x 70 cm, to a depth of approximately 10 cm. The understory treatments were randomly assigned to experimental plots within blocks, which were 10 m apart, with ten and eleven replicates per treatment in Waipara and Tai Tapu, respectively.

2.2 Arthropod sampling protocols

A sample of mulch was removed by hand, to the depth of the soil surface under a 20 cm x 10 cm quadrat in each plot. Each sample was then sieved through a 50 cm circular sieve (aperture size 6mm) on to a 35 mm x 30 mm rectangular tray. All invertebrates in the trays were then placed in labeled plastic bags and individuals in selected taxa were counted in the laboratory. After the mulch sample was removed, all other remaining arthropods were extracted from the soil surface using a modified leaf blower machine which had a nozzle area of $0.02m^2$ which aspirated the samples. It was placed firmly and vertically on the soil surface for 3 seconds. Four consecutive subsamples were taken from the quadrat and the subsamples were pooled. Then the arthropods and other debris were placed in labeled plastic bags and assessed for arthropod abundance in the laboratory. The arthropods collected were identified to either order or family. Data from the two totals were combined and analyzed. The samples were taken from the plots over six periods of 2 days (one date for each study vineyard), beginning March 10 and 12, April 21 and 23, May 28 and 29, July 27 and 28, September 9 and 10 and October 8 and 9, 2009; besides there was an additional sampling day at Tai Tapu (March 24).

As well as the above two sampling methods, the number of European earwigs, *Forficula auricularia* L. (Dermaptera: Forficulidae), were sampled on six dates in Spring: October 8, 15, 22 and 29 and November 6 and 12, 2009. A piece of corrugated cardboard (10 cm wide and 30 cm long) rolled as a cylinder was used as a shelter trap. These were wrapped around the middle of the wine trunks.

2.3 Assessing predation rates

Laboratory reared eggs of the lightbrown apple moth *E. postvittana* (LBAM) were used as sentinel prey to evaluate predation rates in the grapevine canopy in both vineyards. Strips of filter paper (≈ 7 cm^2) containing egg batches comprising 4-19 eggs were stapled to the underside of mature grapevine leaves, one to each vine in every plot. Baits were positioned on the east (Waipara) and south (Tai Tapu) outer sections of the canopy, 20-30cm from the trunk and at an approximate height of 1.60 cm. from the ground. After 24 h, the baits were collected and assessed for predation rate in the laboratory. This was assessed eight and seven times at Tai Tapu and Waipara, respectively from April to November, 2009.

2.4 Soil characteristics

Soil temperature, soil moisture, water infiltration rate, soil biological activity (bait lamina probes and dehydrogenase activity), numbers of earthworms, organic matter and soil nutrients were compared among treatments. Soil moisture, number of earthworms and bait lamina probes were assessed in all blocks. Temperature and water infiltration rate were measured only in three randomly selected blocks each in Waipara and Tai Tapu. Dehydrogenase activity, soil organic matter and soil nutrients were assessed in three other randomly selected blocks at each of the two sites.

Soil temperatures were recorded at a depth of 5 cm, at two-hourly intervals, on iButton^{TM} data loggers (Maxim DS1921G), in three randomly selected blocks from May 14 to November 10, 2009, at Waipara. The dataloggers were positioned 20 cm from the vine trunk parallel to the vine rows.

Soil moisture was measured from one randomly selected location from each plot and the samples were taken on May 13 and November 12, 2009. Soil samples were taken with a soil corer (10 cm height and 3 cm diameter). They were put in plastic bags and immediately transported to the laboratory where they were weighed then dried in an oven at 70ºC for 14 days, and then weighed again. Percentage of moisture in the soil was calculated for each sample.

Water infiltration rate and soil hydraulic conductivity were each measured three times on June 4 and November 19 (Tai Tapu) and on June 5 and November 18, 2009 (Waipara) from three randomly selected blocks from each vineyard with a double ring infiltrometer (Taylor et al., 1991). This consisted of inner and outer rings inserted into the ground. Each ring was supplied with a constant head of water from a Mariotte container by siphon action. The rate of infiltration was determined by the volume of water that infiltrated the soil surface per second. Initial measurements were recorded as soil sorptivity as the soils' pores filled and became saturated. Once soil infiltration rates became constant, the soil was considered saturated and hydraulic conductivity was assessed. The water infiltration rate was calculated in millimeters per second. A mean was calculated from the three readings.

Four earthworm samples were taken in each of the two vineyards on four dates from May to October, 2009, at each vineyard. In each plot, a section of soil 144 cm^2 by 15 cm deep was removed and spread over a plastic sheet where the soil was sorted and all earthworms collected. They were counted and weighed (±0.1 g), and the adults identified to species in the field (only for the samples taken in July, September and October).

Soil biological activity was measured through bait lamina probes and dehydrogenase analysis. Bait lamina probes are strips of rigid plastic, 6 × 162 mm, each with sixteen 2-mm holes drilled into the lower 80 mm of the strip. Bait, which had previously been placed in these holes, comprised cellulose, agar, bentonite and bran, all constituents intended to mimic dead plant material in the soil. The test assumes that soil invertebrates and microorganisms progressively consume or degrade the baits (Kratz, 1998; Jacometti *et al.*, 2007a). In this study the number of baits that were intact or completely removed was counted and they gave a measure of the activity of those organisms in the soil. One probe was inserted into a slit the ground which was prepared with a knife (12 x 2 x 0.2 cm) to avoid any damage to the baits or the strips, but good bait/soil contact was provided. Bait lamina probes were placed in all plots in both vineyards. At Tai Tapu, the probes were placed in the ground on the May 14 and September 24 and removed on September 23 and November 17, 2009, respectively. In Waipara, the probes were inserted on the May 14, July 28, September 17 and October 20 and removed on July 16, September 10, October 16 and November 19, 2009, respectively. The data were standardized by dividing the number of baits removed by month or the fraction of a month.

Soil biological activity was also assessed through dehydrogenase analysis (Alef, 1995). This involved the measurement of total dehydrogenase activity amongst soil microbial flora and fauna as an index of the total oxidative activity and therefore of overall microbial activity of the soil. Fresh soil cores were taken at both sites on December 3 and the procedure was carried out in the laboratory on December 7, 2009.

Total organic matter and the carbon to nitrogen ratio are used as a guide to soil quality and the probable rate of organic matter mineralization. The effect of mulches on soil organic matter was assessed using an Elementar Vario-Max CN Elemental Analyser (Elementar GmbH, Hanau, Germany) which determined total carbon and total nitrogen from three randomly selected blocks from each of the two vineyards on December 3, 2009. Three soil cores 10 cm long by 3 cm in diameter were taken in each block and then the soil was pooled, dried at 30ºC for 2 days and ground to a fine powder. Samples weighing between 512- 598 mg were analyzed for carbon and nitrogen.

The effect of mulches on nutrients in vine foliage was also assessed. Forty leaf petioles were taken from every plot from 3 randomly selected blocks from each vineyard at flowering on the December 10, 2009. Petioles were selected opposite the basal cluster from exposed shoots on the outside of the canopy. These petioles were oven dried at 62ºC overnight and ground to pass through a 1 mm screen and then analyzed using different methods. The nitrogen was analyzed with the Dumas combustion procedure. This involved the nitrate-N being extracted with 2% acetic acid followed by a salicylate colorimetry. The rest of the nutrients were subjected to a nitric acid or hydrogen peroxide digestion followed by ICP-OES.

2.5 Harvest

On April 7 in Waipara and May 18, 2010, in Tai Tapu all grapes were removed by hand from all the vines from the experimental plots. This coincided with the dates on which the rest of the grapes were harvested from the vineyard. The number of bunches per vine was counted, then combined and weighed to obtain the total yield per replicate. Total number of earwigs, LBAM, other predators and *D. tasmanica* were visually assessed per bunch. LBAM damage and *B. cinerea* infection were visually assessed by estimating the percentage damage per bunch. Each bunch was also visually assessed for the percentage of split berries and mean number of small green unfertilised berries. Berry sugar concentrations were measured with an optical refractometer, on four randomly selected bunches per vine from each plot.

2.6 Statistical analysis

Analysis of variance (ANOVA) was used to analyze the dehydrogenase activity, soil organic matter and the effects of mulches on petiole nutrients. For arthropod abundance, egg baiting, earthworm abundance, soil moisture, bait lamina probes, soil temperature and water infiltration rate, repeated measures ANOVA was used. In order to meet the assumption of normality required by these methods, arthropod abundance data were log transformed prior to analysis. Egg baiting data, earthworm abundance, water infiltration rate in Waipara and bait lamina probe data in Waipara were square root transformed. The remaining data sets conformed to a normal distribution, so no transformation was necessary. Normality of the data was confirmed with the Levene test. To determine the differences between the means the Duncan test was used for the egg baiting data, earthworm abundance and soil moisture, and the Tukey test was used for all other soil characteristics. Arthropod abundance data was analyzed with GenStat (Seventh Edition Version 7.1.0.198), and all other data were analyzed with SAS (SAS Enterprise Guide 4.1).

3. Results

3.1 Arthropod abundance and classification

Analysis focused on invertebrates collected in sufficient numbers and depending on their ecological role in the vineyards: Collembola (Hypogastruridae and Paronellidae), Acarina, Psocoptera, Staphylinidae, and Lygaeidae. While Araneae, Formicidae and Diplopoda were present in both vineyards, they were not found in sufficient numbers for analysis.

Hypogastruridae was the most common arthropod group and was found in similar numbers in all treatments (Figure 6.1). Significantly more were found in mulches, compared with bare ground at both Tai Tapu (df=2, F=71.13, P<0.001) and Waipara (df=3, F=78.59, P<0.001), but without any significant effect of mulch type. The numbers decreased at both Tai Tapu (df=6, F=70.33, P<0.001) and Waipara (df=6, F=73.70, P<0.001) over time. A treatment-time interaction was also found at both Tai Tapu (df=12, F=6.66, P<0.001) and Waipara (df=18, F=5.38, P<0.001).

Similar results were found for Paronellidae (Figure 6.1). This family was not as abundant as Hypogastruridae, but was also found in similar numbers in all mulches. As for Hypogastruridae, significantly more Paronellidae were found in mulches compared with bare ground at Tai Tapu (df=2, F=52.94, P<0.001) and Waipara (df=3, F=55.51, P<0.001), again without any effects of mulch type. Similarly, abundance decreased over time at both Tai Tapu (df=6, F=46.58, P<0.001) and Waipara (df=6, F=18.74, P<0.001). A treatment-time interaction was also observed at Tai Tapu (df=12, F=2.36, P=0.028) and Waipara (df=18, F=4.15, P<0.001).

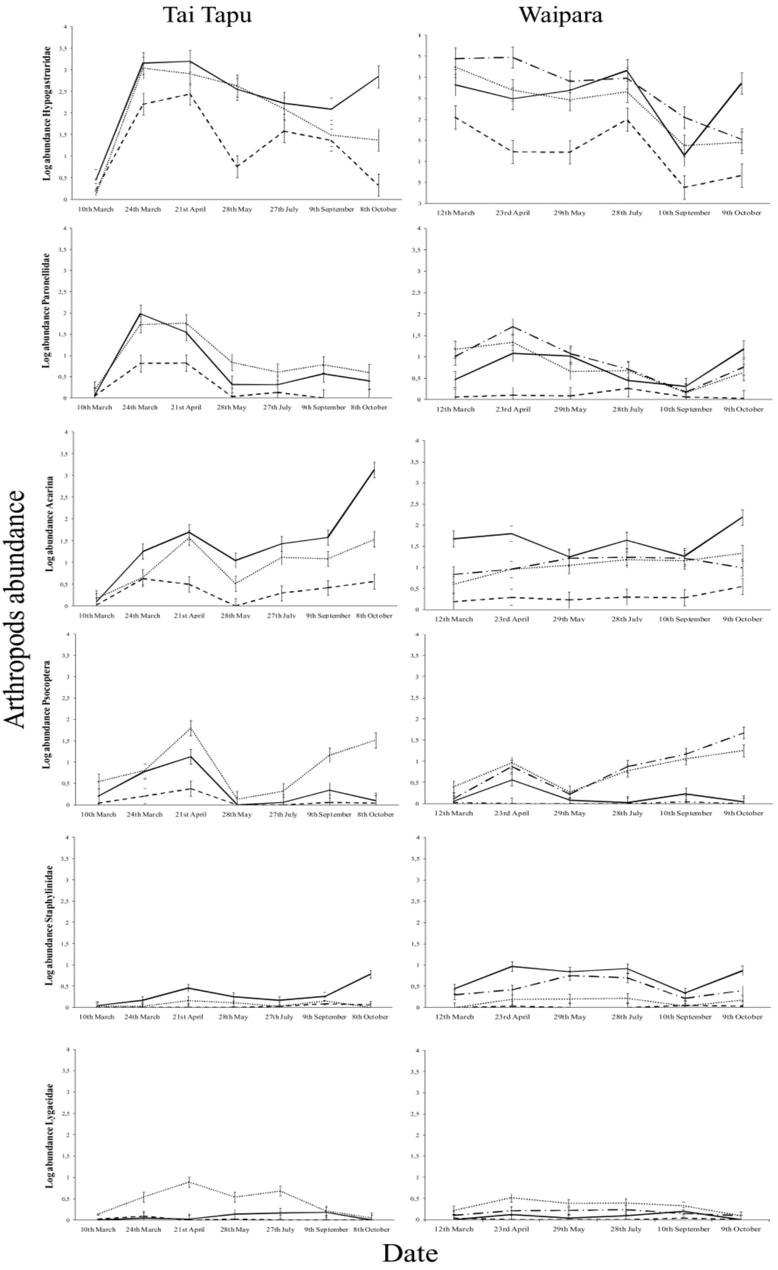

Figure 6.1: Mean abundance of arthropods through the time at both vineyards for the different treatments: grass clippings (—), linseed straw (····), pea straw (−·−) and bare soil (---). Error bars indicate the standard error of difference from log transformed repeated measures ANOVA. Log transformed data are presented.

Various orders of Acarina were found with the majority being Oribatids. These mites were significantly more abundant in the grass clippings treatments at both Tai Tapu (*df*=2, *F*=126.70, *P*<0.001) and Waipara (*df*=3, *F*=134.70, *P*<0.001) (Figure 6.1). There was an increase in numbers found at Tai Tapu (*df*=6, *F*=54.76, *P*<0.001) over time but not in Waipara (*df*=6, *F*=4.63, *P*=0.002) where the numbers were more constant throughout the season. A treatment-time interaction was observed at both Tai Tapu (*df*=12, *F*=11.25, *P*<0.001) and Waipara (*df*=18, *F*=2.31, *P*=0.013).

Psocoptera at Tai Tapu were significantly more abundant in the linseed straw treatment (*df*=2, *F*=94.18, *P*<0.001), while at Waipara most were collected fromboth linseed and pea straw (*df*=3, *F*=82.59, *P*<0.001) (Figure 6.1). In both Tai Tapu (*df*=6, *F*=23.04, *P*<0.001) and Waipara (*df*=6, *F*=23.69, *P*<0.001) there was a drop in numbers in late autumn and an increase in spring. A treatment-time interaction was also found in Tai Tapu (*df*=12, *F*=6.08, *P*<0.001) and Waipara (*df*=18, *F*=9.13, *P*<0.001).

At Tai Tapu, significantly more Staphylinidae were collected in grass clippings (*df*=2, *F*=20.56, *P*<0.001), while in Waipara more were also collected in that treatment and also in the pea straw treatment (*df*=3, *F*=68.93, *P*<0.001) (Figure 6.1). A slight increase in numbers was observed over time in Tai Tapu (*df*=6, *F*=6.60, *P*<0.001) but not in Waipara (*df*=6, *F*=15.95, *P*<0.001). At both Tai Tapu (*df*=12, *F*=5.79, *P*<0.001) and Waipara (*df*=18, *F*=3.94, *P*<0.001) numbers were affected by treatment-time interaction.

Significantly more Lygaeidae were collected in linseed straw in both Tai Tapu (*df*=2, *F*=23.81, *P*<0.001) and Waipara (*df*=3, *F*=15.57, *P*<0.001) (Figure 6.1). Over time there was a decrease in the numbers collected at both Tai Tapu (*df*=6, *F*=6.78, *P*<0.001) and Waipara (*df*=6, *F*=2.92, *P*=0.02). A treatment-time interaction occurred at Tai Tapu (*df*=12, *F*=5.78, *P*<0.001), but not at Waipara.

Various families of the order Araneae were present in the vineyards, mainly in linseed (Tai Tapu and Waipara) and pea straw (Waipara only), with fewer in grass clippings. Diplopoda were collected mainly in linseed (Tai Tapu and Waipara) and pea straw (only Waipara) as well. Slightly higher numbers of Formicidae were found in grass clippings and linseed straw at Tai Tapu compared with bare ground, whereas at Waipara greater numbers were found in all mulches compared with bare ground.

No earwigs were found in any of the cardboard traps at Waipara. However, at Tai Tapu they occurred in every treatment and more were found in the bare ground treatment compared with the other two.

3.2 Assessing predation rates

Although mulches had no effect on the percentage of predation of LBAM eggs in the canopy at either vineyard, predation rates did decrease in both Tai Tapu (*df*=6, *F*=6.67, *P*<0.0001) and Waipara (*df*=5, *F*=29.55, *P*<0.0001) over time. This was especially evident at Waipara where rates dropped ten-fold from the first assessment (51.9% baits consumed) to the last (6.9% baits consumed).

3.3 Soil characteristics

At Waipara, mulches increased mean soil temperature (*df*=3, *F*=5.72, *P*=0.0217) (Table 6.1). There was a treatment-time interaction (*df*=18, *F*=46.92, *P*<0.0001) where early year (May) soil temperatures were lower in the control than the mulch plots, but higher later in the year. Soil temperature range was lower under mulch compared to the control plots (*df*=3, *F*=91.71, *P*=<0.001).

At Tai Tapu, soil moisture was higher under mulch compared to bare ground (*df*=2, *F*=5.68, *P*=0.011) (Table 6.2) but the same effect was not observed at Waipara (Table 6.1). Tai Tapu also showed a time effect (*df*=1, *F*=100,31, *P*<0.001) and a treatment-time interaction (*df*=2, *F*=7.24, *P*=0.003), with a much greater soil moisture increase under mulch compared with bare ground throughout the season. On the contrary, at Waipara soil moisture was only affected by time (*df*=1, *F*=178.16, *P*<0.001), showing a decrease in soil moisture across all the treatments.

Neither vineyard showed an effect of mulches on water infiltration rate, but there was a treatment-time interaction at both: Tai Tapu (*df*=2, *F*=6.51, *P*=0.0314) (Table 6.2); Waipara (*df*=2 *F*=6.51, *P*=0.031) (Table 6.1).

Table 6.1: Effects of mulches on vineyard soil characteristics at the Waipara site.

	Bare soil	Grass clippings	Linseed straw	Pea straw	All mulches
Soil characteristics					
Soil temp variation (ºC) (LSD$^{5\%}$ = 2.512)					
May	10.83	5.33	5.83	5.00	6.75^{w}
June	10.5	5.83	5.83	5.00	6.79^{w}
July	9.83	6.17	6.83	5.17	7.00^{w}
August	14.5	7.67	7.67	6.33	9.04^{x}
September	19.67	9.50	9.33	7.33	11.46^{y}
October	20.17	14.00	16.00	15.33	16.38^{z}
November	19.67	9.33	9.33	8.83	11.79^{y}
All months	15.02^{b}	8.26^{a}	8.69^{a}	7.57^{a}	
Soil moisture (%)					
May	13.86	14.56	15.56	15.65	14.91^{z}
November	8.88	10.32	9.06	10.24	9.62^{y}
All months	11.37^{a}	12.44^{a}	12.31^{a}	12.94^{a}	
Soil water infiltration (mm/s) (sqrt transformed*) (LSD$^{5\%}$=0.2319)					
May	0.354	0.322	0.320	0.518	0.379^{z}
November	0.178	0.516	0.381	0.312	0.347^{z}
All months	0.266^{a}	0.419^{a}	0.351^{a}	0.415^{a}	
Organic matter (%)	2.85^{a}	3.52^{a}	2.79^{a}	3.14^{a}	
C/N ratio	9.08^{a}	9.56^{a}	8.82^{a}	9.64^{a}	
N (%)	1.23^{b}	2.03^{a}	1.10^{b}	1.23^{b}	
P (%)	0.18^{a}	0.14^{a}	0.14^{a}	0.15^{a}	
K (%)	2.73^{a}	3.33^{a}	2.57^{a}	2.63^{a}	
Dehydrogenase activity	49.26^{a}	69.02^{a}	48.06^{a}	73.42^{a}	
Bait lamina probes (baits consumed/day) (sqrt transformed*)					
July	1.913	1.325	1.889	1.660	1.697^{y}
September	1.028	1.124	2.070	1.799	1.505^{y}
October	2.517	2.286	2.214	2.643	2.415^{z}
November	1.758	3.124	3.574	2.664	2.780^{z}
All months	1.804^{a}	1.965^{a}	2.437^{a}	2.192^{a}	
Plant attributes					
Berry sugar concentration	22.84^{b}	23.01^{b}	21.62^{a}	23.36^{b}	
Small berries (%)	12.4^{a}	51.1^{b}	8.9^{a}	20.8^{b}	
Split berries (%)	14.4^{a}	34.9^{b}	9.9^{a}	18.4^{a}	
Yield (g/vine)	2012^{b}	888^{a}	2365^{c}	1636^{b}	

Letters a, b, c denote significant differences found between treatments (*P*<0.05; LSD $^{5\%}$); letters x, y, z denote significant differences found between months (*P*<0.05; LSD $^{5\%}$); LSDs presented by various soil attributes is derived from the mulch*month interaction. *Square root transformed data presented.

Table 6.2: Effects of mulches on vineyard soil characteristics at the Tai Tapu site.

	Bare soil	Grass clippings	Linseed straw	All mulches
Soil characteristics				
Soil moisture (%) (LSD$^{5\%}$=1.499)				
May	20.07	21.39	20.48	20.65^{y}
November	21.65	24.27	24.79	23.57^{z}
All months	20.86^{a}	22.83^{b}	22.63^{b}	
Soil water infiltration (mm/second) (LSD$^{5\%}$=0.0716)				
May	0.040	0.060	0.112	0.070^{z}
November	0.048	0.177	0.044	0.090^{z}
All months	0.044^{a}	0.119^{b}	0.078^{ab}	
Organic matter (%)	3.99^{a}	3.71^{a}	3.77^{a}	
C/N ratio	9.34^{a}	9.22^{a}	9.58^{a}	
N (%)	1.47^{a}	1.43^{a}	1.40^{a}	
P (%)	0.19^{a}	0.16^{a}	0.13^{a}	
K (%)	2.90^{a}	2.53^{a}	3.00^{a}	
Dehydrogenase activity	71.40^{a}	69.62^{a}	59.02^{a}	
Bait lamina probes (baits consumed/day)				
September	1.48	1.69	1.84	1.67^{y}
November	2.58	4.65	3.89	3.70^{z}
All months	2.03^{a}	3.17^{b}	2.86^{ab}	
Plant attributes				
LBAM larva/ bunch	3.36^{b}	1.00^{a}	1.27^{a}	
LBAM damage/ vine	2.56^{b}	1.54^{a}	1.23^{a}	
Percentage of *B. cinerea* infection	1.42^{b}	0.66^{a}	0.96^{a}	

Letters a, b, c denote significant differences found between treatments ($P<0.05$; LSD $^{5\%}$); letters x, y, z denote significant differences found between months ($P<0.05$; LSD $^{5\%}$); LSDs presented by various soil attributes is derived from the mulch*month interaction.

Earthworm biomass showed a significant response to the treatments at both Tai Tapu (*df*=2, *F*=5.06, *P*=0.0130) and Waipara (*df*=3, *F*=5.46, *P*=0.0034) (Figure 6.2). There was also a treatment-time interaction at both vineyards: Tai Tapu (*df*=6, *F*=2.84, *P*=0.0142); Waipara (*df*=9, *F*=2.23, *P*=0.0251). At Tai Tapu, there were significantly more earthworms in linseed in May and July and in grass clippings in October, while at Waipara, there were significantly more in pea straw in July and September and in grass clippings in October. Overall, the number of earthworms found was higher on the mulches compared with the bare soil; this was more noticeable in Tai Tapu. Four earthworm species were found in both vineyards: *Lumbricus rubellus* (Hoffmeister), *Aporrectodea longa* (Ude), *Lumbricus terrestris* L. and *Aporrectodea caliginosa* (Savigny). Another species, *Dendrodilus rubidus* (Savigny), was only observed at Waipara in July and

September but only in bare soil. The most abundant species found at Tai Tapu was *L. rubellus*. In contrast, *A. caliginosa* was found in greatest numbers at Waipara, followed by *L. terrestris* and *D. rubidus.* At both vineyards, the most common species found on bare ground, under pea straw and under grass clippings was *A. caliginosa*, while the most common species found under linseed at both sites was *L. rubellus*.

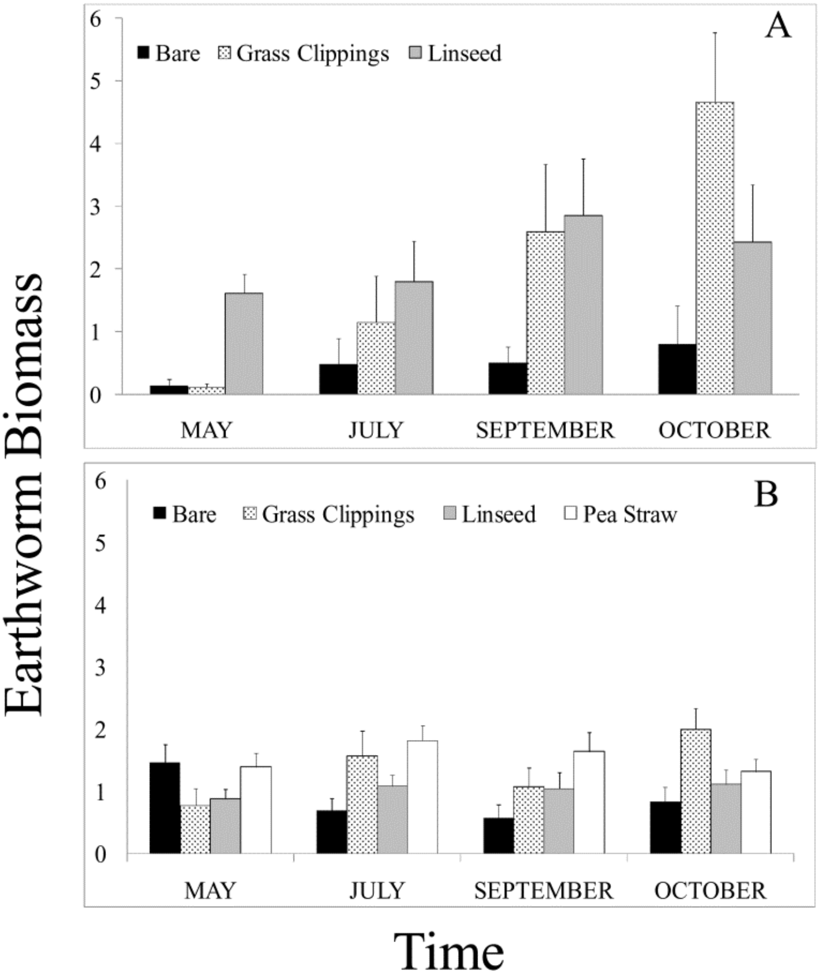

Figure 6.2: Mean earthworm biomass through the time at Tai Tapu (A) and Waipara vineyards (B). Error bars indicate the standard error of difference.

Soil biological activity measured using bait lamina probes was not affected by any treatment at either vineyard, but over time activity did increase at Tai Tapu (*df*=1, *F*=28.78, *P*<0.001) (Table 6.2) and Waipara (*df*=3, *F*=11.64, *P*<0.001) (Table 6.1). No differences were observed in dehydrogenase activity at either vineyard.

The percentage of organic matter in the soil was not affected by the different treatments in Tai Tapu (Table 6.2). At Waipara, although the differences between treatments were not significant, a higher percentage of organic matter was observed under grass clippings and pea straw treatments (Table 6.1). Mean C: N ratio of the soil was not significantly affected by any of the mulch treatments.

Statistical differences only occurred for nitrogen (*df*=3, *F*=15.62, *P*=0.0010), boron (*df*=3, *F*=4.21, *P*=0.0461) and nitrate (*df*=3, *F*=10.85, *P*=0.0034) in Waipara. No statistical significant differences were observed for the other nutrients in leaf petioles samples taken at Waipara (Table 6.1), and no significant differences at all were observed in samples from Tai Tapu (Table 6.2).

3.4 Harvest

At Waipara, significant differences were found between treatments for mean Brix (*df*=3, *F*=4.33, *P*=0.013), number of small berries per bunch (*df*=3, *F*=14.64, *P*<0.001), mean number of split berries per bunch (*df*=3, *F*=10.23, *P*<0.001) and total yield (*df*=3, *F*=6.57, *P*=0.002) (Table 6.1). At Tai Tapu, significant differences were found between treatments for number of LBAM larvae per bunch (*df*=2, *F*=4.42, *P*=0.026), percentage of LBAM damage per vine (*df*=2, *F*=53.48, *P*=0.005) and percentage *B. cinerea* infection per vine (*df*=2, *F*=5.86, *P*=0.018) (Table 6.2). No other statistical differences were found in either vineyard.

4. Discussion

4.1 Arthropod abundance and classification

Results of this study indicate that mulches are beneficial to arthropod populations compared with bare ground. The most abundant group was Collembola which has an important ecosystem function as a detritivore and consumes and processes considerable amounts of litter (Hamel et al., 2007). In a study by Mathews et al. (2002) to compare different mulches in an apple orchard in West Virginia (USA), Collembola were also the dominant edaphic group. The use of linseed straw, grass clippings and pea straw (Waipara only) as mulches increased the numbers of Hypogastruridae and Paronellidae compared with bare ground at both vineyards. Other studies have also shown the benefits of mulching for detritivorous arthropods (Mathews *et al.*, 2002; Brown and Tworkoski, 2004). In contrast, Thomson and Hoffmann (2007) studied the effects of ground covers on the abundance of arthropods in vineyards in Australia and they found lower numbers of Collembola under straw mulch compared with bare ground and under compost.

Oribatid mites were the main taxon collected from the subclass Acarina. These mites live in the soil organic horizon and are often used as a bioindicator (Behan-Pelletier, 1999). These mites were most abundant in the grass clippings in the current work. The pattern followed at the two vineyards was different, showing an increase in Tai Tapu but not in Waipara.

Members of the family Staphylinidae are mainly known as non-specific predators and can be used as bioindicators of the environmental status (Bohac, 1999). In this study they were the most commonly found predator group. They had the highest abundance in grass clippings at both vineyards and also they were at high densities in pea straw at Waipara, indicating the advantages of mulches compared with bare ground. Other studies have also found that the addition of mulches benefits this group of insects (Mathews *et al.*, 2002; Brown and Tworkoski, 2004; Mathews *et al.*, 2004). However, Thomson and Hoffmann (2007) showed that Staphylinids were not affected by the addition of mulches.

Psocoptera were mainly found in linseed straw at both vineyards and also in pea straw at Waipara. The higher numbers found under linseed straw could be a consequence of the main group of predators (Staphylinidae) being found mostly on grass clippings and pea straw. Psocoptera could have been preyed upon by Staphylinidae.

The Lygaeidae collected in the study were also affected by mulch type, being found mainly in linseed straw at both vineyards. These arthropods are also primarily herbivores and therefore high numbers in the linseed treatment may also be a consequence of the higher abundance of Staphylinidae for the grass clippings and pea straw.

In contrast with other studies in which Formicidae and Araneae dominated the predator complex (Mathews et al., 2002, 2004), this study found both in low. Araneae were influenced by the addition of mulch and were mostly found in linseed straw at both vineyards, as well as in pea straw at Waipara. Previous studies have shown the positive effects of mulches on Araneae (Thomson et al., 2007). The Formicidae, on the other hand, followed a different pattern at each vineyard. At Tai Tapu, although more ants were found under the mulch treatments, there was not a strong difference compared with the number found on bare ground. At Waipara, though, this difference was much greater.

Studies have demonstrated that earwigs have the potential to be considered an important predator of canopy pests (Suckling *et al.*, 2006; Frank *et al.*, 2007). In this study no earwigs were found at Waipara, but they were present in every treatment at Tai Tapu. Maher and Logan (2007) found that the number of earwigs in an organic orchard in midsummer in New Zealand was significantly greater than in a conventional orchard. This is supported by Waipara data (a conventionally managed site) and Tai Tapu, being a low inputs vineyard. Across the different treatments at Tai Tapu earwigs showed a clear preference for bare ground. In a study carried out by Burnip et al. (2002) more earwigs were trapped in the herbicide treatment compared with the straw treatment.

4.2 Assessing predation rates

None of the mulch treatments had any effect on percentage of LBAM predation on the canopy at either vineyard. A study by Suckling et al. (2006) in Canterbury, New Zealand, found that predation rates in apple trees on seeded leafroller egg batches were high from December to March and also higher when pea straw was used compared with herbicide-treated plots. On the other hand, Mathews et al. (2004) found in an apple orchard in West Virginia (USA) that higher percentages of *Cidia pomonella* (L.) (Lepidoptera: Tortricidae) larvae were predated by ants and carabids in herbicide-treated plots compared to compost or synthetic mulches. Since our study took place from April to November, and thus during the coldest period of the year in the area (southern hemisphere), predation rates may have been low, making it difficult to detect differences between the treatments.

4.3 Soil characteristics

The lower mean temperatures in the mulch plots were typical of those found in other studies (Buckerfield and Webster, 1996; Mathews et al., 2002; McIntyre et al., 2003). The mulches also reduced the temperature range at the soil surface. This has also been recorded in other studies (Jacometti et al., 2007b).

Protecting the surface of the soil with mulch reduces water loss by evaporation (Buckerfield and Webster, 2002). Other works have shown increased soil moisture under mulch

(Buckerfield and Webster, 1996; Mathews et al., 2002; McIntyre et al., 2003). Our study supports this at the Tai Tapu vineyard but not at Waipara, where soil moisture was not affected by the mulch treatments. This may be due to the different soil types in the two vineyards, since Tai Tapu has a clay loam soil, while Waipara has a silt loam.

Looking at water filtration rate, a previous study (McIntyre et al., 2003) has shown this to increase with mulching. However, our study showed no effect of any of the mulch treatments on infiltration rate. A possible explanation could be that the period when our study was carried out was no longer enough for observing these differences in the infiltration rate.

Earthworms provide several ecosystem services including increasing topsoil fertility, providing food for predators and recycling organic materials (Boyer and Wratten, 2010). Similar to other studies (Buckerfield and Webster, 1996; Thomson and Hoffmann, 2007), our study indicated that earthworms were more abundant under the mulch. Both Tai Tapu and Waipara showed a trend over time, but under different mulch treatments. At Tai Tapu more earthworms were found in linseed in late autumn and at the end of the summer. At Waipara, more earthworms were found under pea straw in the same periods. However, at the beginning of spring earthworms were significantly more abundant in grass clippings in both vineyards. *Lumbricus rubellus*, *A. longa*, *L. terrestris* and *A. caliginosa* were present at both vineyards, while *D. rubidus* was only present in Waipara, and in low numbers.

A thorough understanding of soil processes requires knowledge and consideration of soil fauna (Hamel et al., 2007). A good way to achieve this is the bait lamina test system used in agricultural research to measure the influence of different cropping patterns on the feeding activity of soil arthropods (Kratz, 1998). In our study, although soil biological activity increased over time, it was not affected by the different treatments. These results contrast with those of Jacometti et al. (2007a, b) who found that mulches (aerobic and anaerobic marc and paper treatments) significantly increased soil biological activity. In our study, dehydrogenase activity was also not affected by the treatments in either vineyard.

The percentage of organic matter in the soil was not significantly affected by the treatments at either of the vineyards. However, the Waipara vineyard did tend to show a higher percentage of organic matter under grass clippings and pea straw. The C:N ratio of the soil was also unaffected by the treatments, in contrast to a study by Jacometti et al. (2007b), where mulches had a significant effect on mean C:N ratio of the soil.

The analysis of leaf petioles did not show any significant differences in the nutrients obtained across the treatments in Tai Tapu. In Waipara only nitrogen, potassium, boron and nitrate were affected.

4.4 Harvest

At the time of harvest the attributes significantly affected by the treatments were quite different in the two vineyards. At Waipara mean Brix level was lower in linseed straw compared with the control and the other two mulch treatments. This differed with the results of Jacometti et al. (2007b) who found that the Brix was elevated under the mulches. In addition, vines at Waipara that experienced the grass clippings treatment produced a higher number of small berries, more split berries per vine and a lower total yield, while vines with the linseed treatment produced a higher total yield. At Tai Tapu, none of the aforementioned attributes were significantly affected by the treatments. Instead, a greater number of LBAM larvae per bunch, a higher percentage of LBAM damage per vine and an elevated percentage of *B. cinerea* infection were found for the control. These results coincide with those of Jacometti et al. (2007a, b) who observed a reduction in *B. cinerea* infection when aerobic marc, anaerobic marc and paper were compared to a no-mulch control.

5. Concluding remarks

This work was carried out in the context of increasing global emphasis on policies and practices supporting sustainable food and beverage production. One of the features of modern agriculture is its high reliance on agrochemicals. Along with on-farm fuel use and off-farm transport of food, these energy inputs are unsustainably high. For example, in the USA in 1940, 1 calorie put into farming delivered 2.3 calories ‘to the plate’. In 2000, the figures were 7.3 calories in, 1 out (Heller and Keoleian, 2000). More recently the United Nations has criticised modern farming and strongly advocates an agroecological approach (De Schutter, 2010). This manuscript, therefore, is based on deploying renewable resources in viticultural management and contributes strongly to the above global issues.

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General discussion

The different experiments carried out in this work have deepened the knowledge of how plant-based resources can improve conservation biological control programs as well as how they can enhance some ecosystem services in vineyards.

Previous work showed that the genus *Orius* established and maintained well on *Lobularia maritima*, alyssum, during times between crops (Alomar *et al.*, 2008). Additionally, alyssum is a perennial Mediterranean herb with an extended flowering period and, therefore, was selected for the first approaches of this work. In **Chapter 1** I demonstrated that, in fact, alyssum can provide necessary resource subsidies for the maintenance of *O. majusculus*. The predator was able to survive on alyssum without prey for a period that was not different from that obtained on green bean pods with prey, which reflects the benefits of using an entire plant that contains subsidies such as pollen or nectar. Other authors have also shown the benefits on fecundity and longevity of using flowers over green bean pods and other plan structures for a congener, *O. insidiosus* (Mendes *et al.*, 2005; Carvalho *et al.*, 2010). Moreover alyssum without prey supported some reproduction of *O. majusculus*, although the maximum reproduction on this plant by this bug was only possible with the addition of prey to the diet. When prey was added to the diets, not only the fecundity obtained on alyssum plants was much greater than the control, the green bean pods, but it also extended for a much longer period. These findings correspond with other works where *Orius* spp. fecundity and longevity improved with the addition of prey to the diet (Funao and Yoshiyasu, 1995; Cocuzza *et al.*, 1997). Additionally, the results of the host-plant change experiment showed that females laid eggs on both plants when they were supplemented with prey, independently of their prior experience in the colony source. This latter result reinforces the importance of supplementing *O. majusculus* diets with prey in order to get full reproductive output. The fact that *O. majusculus* oviposition on alyssum does not seem to be influenced by prior experience to another plant is interesting: in the case that commercial releases of the predator were needed in the crop, the use of alyssum as an insectary plant would probably assure the establishment of *O. majusculus*. The results obtained with these experiments strongly indicate that the use of alyssum as an insectary plant might be an effective technique to conserve the predatory bug *O. majusculus* in the fields during times of prey scarcity.

The use of plant-based resources to conserve natural enemies in or around fields is considered to be an important tool within agroecosystems. However an increase in biodiversity does not always represent a benefit but also can entail several risks for the crops (Gurr *et al.*, 2005). Our previous results showed that alyssum can maintain *O. majusculus* and this plant was selected as a candidate companion plant to conserve this predator. Nevertheless, the extent to which this plant could promote other pests as well was unknown. *Orius* spp. is known to coexist with its main prey, thrips, in several weeds (Silveira *et al.*, 2005; Atakan and Tunc, 2010) but the interactions among each other are not well studied. Therefore, in **Chapter 2**, I investigated the predation exerted by *Orius* spp. on thrips on alyssum, but also how the predation on this prey affects the reproduction of the predator. Two *Orius* species, *O. majusculus* and *O. laevigatus,* were able to reduce thrips number at two densities on either alyssum plants or green bean pods in microcosm conditions. In other studies these two *Orius* spp. have effectively preyed on thrips on green bean pods too (Riudavets and Castane, 1998; Tommasini *et al.*, 2004). Nonetheless, while the efficacy of *O. majusculus* to reduce thrips numbers was not affected by the plant species, the efficacy of *O. laevigatus* to reduce thrips on alyssum was lower than on green bean pods. As a result, *O. majusculus* may represent a better option to be used in conservation biological control programs with alyssum as companion plant. Under microcosm conditions and over a longer period of time I could confirm that alyssum is a good reproductive host for thrips too, where it can successfully reproduce. However thrips populations were reduced with *O. majusculus* presence. The predator also reproduced and established on alyssum plants but showed much better performance when fed on *Ephestia kuehniella* eggs than when preying on thrips, as it has been observed for *O. majusculus* and other *Orius* spp. (Kiman and Yeargan, 1985; Tommasini *et al.*, 2004). This is, however, not surprising due to the high quality nutrition provided by those eggs (Cohen, 1989). Thrips predation allowed some reproduction of *O. majusculus*, although the nutrition provided by this prey does not seem very different from the plant itself. In semi-field conditions thrips numbers were lower with high *O. majusculus* densities although the predator did not significantly reduce thrips populations. These results contrast with others where *O. majusculus* reduced thrips populations in greenhouse crops and ornamentals (Blaeser *et al.*, 2004; Fitzgerald, 2006). Nevertheless, *O. majusculus* successfully reproduced on alyssum and dispersed to the lettuce plants. Even though further research is recommended in semi-field conditions, the provision of alyssum plants offers scope to *O. majusculus* establishment without favoring thrips populations and can therefore be selected as a candidate insectary plant for the conservation of *Orius* spp.

Plant subsidies can affect predator's fitness in different ways. In **Chapter 3**, I evaluated how plants that differ in their accessibility to nectar and pollen sources affect the performance of another *Orius* spp, *O. insidiosus*, and whether an increase in plant diversity benefits the fitness of

O. insidiosus. Specifically, I investigated how the exploitation of plant-based resources such as pollen or nectar can affect the survival, reproduction, and nutritional status of *O. insidiosus*. Three species, alyssum, buckwheat and phacelia, were selected because they are among the most commonly used plants in conservation biological control programs (Fiedler *et al.*, 2008). Two other plants were selected according to their floral or extrafloral subsidies access: faba bean, which possess extrafloral nectaries, and chamomile, whose nectaries are hidden in a capitulum (Patt *et al.*, 1997). The different plant species affected the survival, reproduction, and nutrient status of *O. insidiosus*, however the access to the floral resources or extrafloral nectar influenced only the survival but not the reproductive output or the storage of nutrients on *O. insidiosus*. Among the plant species used, phacelia elicited somewhat better survival and fecundity, and females on faba bean had the highest levels of glycogen. The access to the floral or extrafloral resources caused a higher percentage of survival and a lower percentage of missing females, which indicates that *O. insidiosus* is not only sucking phloem but also benefits from consuming some of the flower subsidies. While the plant species seemed to have only immediate effects on *O. insidiosus* survival, the access to the floral resources or extrafloral nectar had marginal effects on the posterior survival of *O. insidiosus*, with a slightly longer longevity found on females that had been on plants with exposed plant subsidies. An increase in plant diversity did not produce differences in survival. However, while longevities on bean pods in the first experiment were extremely low, *O. insidiosus* females survived much longer when directly confined on these plants. It highlights the benefits of feeding on plant resources such as pollen or nectar compared with only green bean pods for *O. insidiosus*. As similarly found in chapter 1 for *O. majusculus*, it shows the importance of nectar and pollen for conservation of *Orius* spp. The fecundity of *O. insidiosus* greatly varied depending on the context. Faba bean was the least preferred oviposition substrate when plants were offered in a no-choice situation; however, it had the greatest number of eggs when plants were offered as a mixture. Therefore, these results show that *O. insidiosus* may consider faba bean a good oviposition site but only when there are other high quality resources available for its offspring. This is not surprising since some authors have found that *O. insidiosus* prefers to oviposit on certain specific plants (Coll, 1996; Lundgren and Fergen, 2006) or even that plant species is more important for this predator than prey availability for oviposition site selection (Seagraves and Lundgren, 2010). Overall, these findings confirm that there are multiple benefits of plants for *O. insidiosus* but they depend on the context in which they are used. It is, therefore, important to identify the most appropriate food plant species that could be used for each natural enemy under different conservation biological control programs.

Plants are complex environments where insects not only feed but also oviposit, mate, refuge and interact with other arthropods. In order to know to what extent predatory Hemiptera are influenced in their reproductive decisions by the presence of other intraguild competitors, the reproduction of *O. insidiosus* and *Nabis americoferus* alone or when combined was evaluated in **Chapter 4**. Specifically, I tested how these bugs alter their oviposition intensity and placement of eggs on a plant in the presence of competitors. In soybean, Clements and Yeargan (1997), showed that these two predators were found on different plant parts and thus a minimal interaction would be expected. However, this study showed that both predatory bugs are able to perceive the presence of competitors on bean plants. Both predators laid more eggs in the presence of the other species compared to when they were exposed to conspecifics: *O. insidiosus* laid slightly more eggs on leaves and petioles whereas *N. americoferus* laid much more eggs on petioles and petiolules. When they were exposed to only conspecifics, *O. insidiosus* laid eggs on the upper part of the plant whereas *N. americoferus* laid eggs equally throughout the height of the plant. When both species were present, *N. americoferus* laid more eggs on the upper quarter of the plant whereas *O. insidiosus* was marginally more likely to lay eggs lower on the plant. It contrasts with another study that showed that when *N. roseipennis* and *O. insidiosus* were presented together on soybean, *O. insidiosus* oviposited on the top of the plant whereas the former preferred the mid-section of the plants (Isenhour and Yeargan, 1982). The findings of this study indicate that these two predatory bugs can detect the presence of one another and that their reproductive decisions are possibly influenced by their intraguild interactions.

In highly disturbed agroecosystems as are annual crops, beneficial insects must refuge in the surrounding vegetation during periods between crops. Once the new crop is sown, those insects must colonize the fields from their refuges. The identification of these refuges is, therefore, important in order to conserve them near the adjacent crops, but this is not always a straightforward task. This difficulty is in part due to the lack of a technique that can be easily used in field studies where large numbers of insects must be analyzed. In this work, the development of plant specific primers that can be easily identified within insects with a simple PCR is proposed, using tomato as a model plant. Specifically, in **Chapter 5** I demonstrated that tomato plant DNA can be detected immediately after feeding in all individuals of insects with different feeding behaviors such as sucking (the predator *Macrolophus pygmaeus*) or chewing (the larvae of the moths *Tuta absoluta* and *Helicoverpa armigera*). Digestion rate, however, depended on feeding type, and longer half-lives were obtained within chewing insects. These results differed from others where longer detection periods were found within sucking insects compared with chewing ones (Greenstone *et al.*, 2007; Hosseini *et al.*, 2008). Moreover, tomato plant DNA was also identified from field collected insects, with higher percentages of detection on females and nymphs than on males. A comparison of our results with those obtained by Moreno-Ripoll (2009), where they detected prey within the insects, showed a simultaneous detection of plant and prey within the same individual, mainly in nymphs. This simultaneous detection of plant and prey indicates dietary mixing and confirms a model that suggests that plant feeding is essential for predation (Sinia *et al.*, 2004). These findings indicate that the development of markers for the screening of possible insectary plants would allow the identification of the plants where the insects had been feeding. Recently, other authors have also identified plant DNA within soil-living insects larvae with this technique (Staudacher *et al.*, 2011).This information would be very useful to understand natural enemy colonization of crops from surrounding vegetation.

Apart from the fact that plant-based resources may contribute to the conservation of natural enemies, there are other benefits to the crops that are related with the use of those plant-based resources, such as the ecosystem services they can provide. In **Chapter 6** I compared the usefulness of several mulches (grass clippings, pea straw and linseed straw) to enhance ecosystem services in vineyards when compared with bare soil. Specifically, I evaluated mulch effects on soil invertebrate's biodiversity as well as on the enhancement of several services in the soil in two contrasting vineyards. Overall, the numbers of arthropods and earthworms found in both vineyards were higher under mulches compared with the bare ground, which was similar to other studies (Buckerfield and Webster, 1996; Brown and Tworkoski, 2004; Thomson and Hoffmann, 2007). The presence of mulches reduced soil temperature fluctuations as well as increased soil moisture at one of the vineyards, which was not different from other studies (Mundy and Agnew, 2002). At both vineyards, predation of light brown apple moth eggs in the canopy was not different among treatments. It differed from that obtained by Suckling (2006) where the percentage of predation was higher when pea straw was used, compared with herbicide-treated plots. Others works have also obtained higher predation rates in the canopy when mulches are deployed (Mathews *et al.*, 2002; Brown and Tworkoski, 2004). However, the fact that the experiment was carried out during autumn, the time of year arthropods are less active, may have diminished this percentage. Attributes measured at the time of harvest were affected by the treatments and they were quite different among vineyards. Despite these differences, the mulches resulted as a whole in an improvement with respect to the bare soil. Overall, the results varied with vineyard location but these findings have proved the potential of mulches for enhancing several ecosystem services in vineyards.

It can be concluded that, overall, the results obtained during this project strongly indicate that the use of plant-based resources may ameliorate the application of conservation biological control programs and ecosystem services in crops, although these plants must be selected and used with appropriate care.

Conclusions

Conclusions to **Chapter 1**:

- The use of *L. maritima*, alyssum, without prey ensures the maintenance and some reproduction of *O. majusculus*; however, to achieve the maximum reproductive output of *O. majusculus* on alyssum, the presence of prey is necessary. Overall these results demonstrate that alyssum could provide the necessary resources for the conservation of this predatory bug in the field during times of prey scarcity.
- Alyssum clearly improves the survival and fitness of *O. majusculus* in relation to green bean pods, especially when prey is added to the diet. It highlights the benefits of using a flowering plant that possess pollen and nectar over a simple structure, which is a green bean pod.
- The host-plant change does not affect *O. majusculus* reproduction in the presence of prey. It would imply that mass reared *O. majusculus* could establish on alyssum once released in the field whenever prey is available.

Conclusions to **Chapter 2**:

- *Orius majusculus* and *O. laevigatus* effectively prey on thrips on alyssum under microcosm conditions. However, in a semi-field situation, the different *O. majusculus* densities do not affect thrips populations, that are not different among treatments.
- Under both microcosm and semi-field conditions, *O. majusculus* is able to reproduce and establish feeding on thrips. However, the results suggest that thrips are not a very nutritious food source for *O. majusculus* compared with other preys such as *E. kuehniella* eggs.
- These findings indicate that alyssum allows the establishment of *Orius* spp. as well as the control of thrips by this predatory genus. Moreover, the predators are able to disperse to the crop at both high and low *O. majusculus* densities. As a result, alyssum could be selected as an insectary plant for the conservation of this predator in the field.

Conclusions to **Chapter 3**:

- Plant species greatly varies in their benefits for *O. insidiosus* and each species affects the reproduction, survival and nutritional status of the predator in a different way; this variability is driven in part by the access to the floral or extrafloral resources of the plants.
- The increase in plant diversity allows *O. insidiosus* to optimize its reproductive output by means of exploiting several plant-based resources such as food and oviposition sites. Some plants can be a suitable oviposition substrate for *O. insidiosus* but only when additional protein or lipid sources are available.
- *Orius insidiosus* responds differently to plant species as well as to the access to floral and extrafloral resources and, thereby, it is important to choose the most appropriate plant for each conservation program. These findings underline that plant mixtures can have positive effects on the fitness and performance of *O. insidiosus*.

Conclusions to **Chapter 4**:

- Both predatory bugs, *O. insidiosus* and *N. americoferus*, can detect the presence of one another and both modified their reproductive decisions depending on the presence or absence of their competitors.
- *Nabis americoferus* greatly increases its oviposition intensity and its preference for certain plant structures in the presence of its competitor whereas *O. insidiosus* only lays slightly more eggs but in the same plant structures in the presence of the other predator. In contrast, *O. insidiosus* marginally modifies its preferences for different plant strata in the presence of the other predator while *N. americoferus* does not.

Conclusions to **Chapter 5**:

- The identification of tomato DNA within insects is possible with the developed markers that in addition are tomato specific.
- These markers allow the detection of the ingested tomato DNA immediately after feeding within all the individuals and all the insect species studied independent of the feeding behavior of the species. The plant DNA half life, however, varies among the different arthropods depending on the feeding type and is longer for the chewing insects.
- The designed markers are also able to detect ingested tomato DNA within field collected insects, with higher percentages of detection on females and nymphs than males. The simultaneous detection of plant and prey in some field-collected individuals suggests dietary mixing in this omnivorous predator.

Conclusions to **Chapter 6**:

- The appropriate deployment of a range of organic mulches ameliorates environmental extremes and enhances a range of ecosystem services in vineyards, as well as improves grape quality and yield.
- Overall, more arthropods (predators among others) and earthworms are found in the mulches compared with the bare ground at both vineyards. Light brown apple moth larval populations and associated damage during harvest in one of the vineyards are lower where mulches had been used, as is botrytis incidence. However, there is no effect on light brown apple moth eggs predation in the vineyard canopy at either vineyard.
- Although the results obtained vary depending on vineyard location and mulch type, these findings suggest that an appropriate use of mulches below vines has several benefits and merits further exploration.

References

Adler, L.S. (2000) The ecological significance of toxic nectar. *Oikos* 91 (3), 409-420.

Agnew, R.H., Mundy, D.C., Spiers, T.M. and Greven, M.M. (2005) Waste stream utilisation for sustainable viticulture. *Water Science and Technology* 51 (1), 1-8.

Agusti, N., Bourguet, D., Spataro, T., Delos, M., Eychenne, N., Folcher, L. and Arditi, R. (2005) Detection, identification and geographical distribution of European corn borer larval parasitoids using molecular markers. *Molecular Ecology* 14 (10), 3267-3274.

Agustí, N., de Vicente, C. and Gabarra, R. (2000) Developing SCAR markers to study predation on Trialeurodes vaporariorum. *Insect Molecular Biology* 9 263-268.

Agusti, N., De Vicente, M.C. and Gabarra, R. (1999) Development of sequence amplified characterized region (SCAR) markers of Helicoverpa armigera: a new polymerase chain reaction-based technique for predator gut analysis. *Molecular Ecology* 8 (9), 1467-1474.

Agustí, N. and Gabarra, R. (2009) Effect of adult age and insect density of *Dicyphus tamanini*i Wagner (Heteroptera: Miridae) on progeny. *Journal of Pest Science* 82 (3), 241-246.

Agusti, N., Shayler, S.P., Harwood, J.D., Vaughan, I.P., Sunderland, K.D. and Symondson, W.O.C. (2003) Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. *Molecular Ecology* 12 (12), 3467-3475.

Agustí, N., Unruh, T.R. and Welter, S.C. (2003) Detecting *Cacopsylla pyricola* (Hemiptera: Psyllidae) in predator guts using COI mitochondrial markers. *Bulletin of Entomological Research* 93 (179-185),

Alauzet, C., Dargagnon, D. and Hatte, M. (1992) Production of *Orius majusculus* (Predatory Heteroptera of the family Anthocoridae). *Entomophaga* 37 (2), 249-252.

Albajes, R. and Alomar, O. (2004) Facultative predators. pp. 818-822 in Capinera, J.L. (Ed) Encyclopedia of Entomology, vol. 2. Dordrecht, The Netherlands, Kluwer Academic Publishers.

Albajes, R., Castañé, C., Gabarra, R. and Alomar, O. (2006) Risks of plant damage caused by natural enemies introduced for arthropod biological control. pp. 132-144 in Bigler, F., Babendreier, D. & Kuhlmann, U. (Eds) Environmental Impact of Invertebrates for Biological Control of Arthropods: Methods and Risk Assessment. Wallingford, UK, CAB International.

Alef, K. (1995) Dehydrogenase activity. In: Methods in Applied Soil Microbiology and Biochemistry. Alef, K., Nannipieri, P. 228-231

Alomar, O. and Albajes, R. (2003) Habitat management for conservation of the native predator *Macrolophus caliginosus*. *Landscape management for functional biodiversity* 26 (4), 4-7.

Alomar, O., Arno, J. and Gabarra, R. (2008) Insectary plants to enhance the biological control of *Nasonovia ribisnigri* and *Frankliniella occidentalis* in lettuce. *Bulletin OILB/SROP* 34: 9-12.

Alomar, O., Gabarra, R., Gonzalez, O. and Arno, J. (2006) Selection of insectary plants for ecological infrastructure in Mediterranean vegetable crops. *Bulletin OILB/SROP* 29 (6), 5-8.

Alomar, O., Goula, M. and Albajes, R. (2002) Colonisation of tomato fields by predatory mirid bugs (Hemiptera : Heteroptera) in northern Spain. *Agriculture Ecosystems & Environment* 89 (1-2), 105-115.

Alomar, O. and Wiedenmann, R.N. (1996) Zoophytophagous Heteroptera: Implications for life history and integrated pest management. Lanham, MD.

Alvarado, P., Balta, O. and Alomar, O. (1997) Efficiency of four heteroptera as predators of *Aphis gossypii* and *Macrosiphum euphorbiae* (Hom.: Aphididae). *Entomophaga* 42 (1-2), 215-226.

Ambrosino, M.D., Luna, J.M., Jepson, P.C. and Wratten, S.D. (2006) Relative frequencies of visits to selected insectary plants by predatory hoverflies (Diptera : Syrphidae), other beneficial insects, and herbivores. *Environmental Entomology* 35 (2), 394-400.

Andow, D.A. (1991) Vegetational diversity and arthropod population response. *Annual Review of Entomology* 36: 561-586.

Araj, S.E., Wratten, S., Lister, A. and Buckley, H. (2009) Adding floral nectar resources to improve biological control: potential pitfalls of the fourth trophic level. *Basic and Applied Ecology* 10 (6), 554-562.

Arnó, J., Roig, J. and Gabarra, R. (2002) Evaluación de variedades de lechuga resistentes al pulgón *Nasonovia ribis-nigri Catalunya Rural i Agraria* 94 34-37.

Arnó, J., Roig, J. and Riudavets, J. (2008) Evaluation of *Orius majusculus* and *Orius laevigatus* as predators of *Bemisa tabaci* and estimation of their prey preference. *Biological Control* 44 (1), 1-6.

Atakan, E. (2010) Influence of weedy field margins on abundance patterns of the predatory bugs Orius spp. and their prey, the western flower thrips (Frankliniella occidentalis), on faba bean. *Phytoparasitica* 38 (4), 313-325.

Atakan, E. and Tunc, I. (2010) Seasonal abundance of hemipteran predators in relation to western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) on weeds in the eastern Mediterranean region of Turkey. *Biocontrol Science and Technology* 20 (8), 821-839.

Avilla, J., Albajes, R., Alomar, O., Castane, C. and Gabarra, R. (2004) Biological control of whiteflies in protected vegetable crops. In: Biocontrol in Protected Culture. K.M. Heinz, R.G. Van Driesche and M.P. Parrella. Ball Publishing, Batavia, IL

Baez, I., Reitz, S.R. and Funderburk, J.E. (2004) Predation by *Orius insidiosus* (Heteroptera: Anthocoridae) on life stages and species of *Frankliniella occidentalis* flower thrips (Thysanoptera:Thripidae) in pepper flowers. *Environmental Entomology* 33 (3), 662-670.

Baggen, L.R. and Gurr, G.M. (1998) The influence of food on *Copidosoma koehleri* (Hymenoptera : Encyrtidae), and the use of flowering plants as a habitat management tool to enhance biological control of potato moth, *Phthorimaea operculella* (Lepidoptera : Gelechiidae). *Biological Control* 11 (1), 9-17.

Baggen, L.R., Gurr, G.M. and Meats, A. (1999) Flowers in tri-trophic systems: mechanisms allowing selective exploitation by insect natural enemies for conservation biological control. *Entomologia Experimentalis et Applicata* 91 (1), 155-161.

Baker, H.G. and Baker, I. (1973) Amino-acids in nectar and their evolutionary significance. *Nature* 241 (5391), 543-545.

Baldi, I., Lebailly, P., Jean, S., Rougetet, L., Dulaurent, S. and Marquet, P. (2006) Pesticide contamination of workers in vineyards in France. *Journal of Exposure Science and Environmental Epidemiology* 16 (2), 115-124.

Barbosa, P. (1998) Conservation Biological Control. Barbosa, P. San Diego: Academic Press.

Barre, P., McKenzie, B.M. and Hallett, P.D. (2009) Earthworms bring compacted and loose soil to a similar mechanical state. *Soil Biology & Biochemistry* 41 (3), 656-658.

Beckman, N. and Hurd, L.E. (2003) Pollen feeding and fitness in praying mantids: The vegetarian side of a tritrophic predator. *Environmental Entomology* 32 (4), 881-885.

Begum, M., Gurr, G.M., Wratten, S.D., Hedberg, P.R. and Nicol, H.I. (2006) Using selective food plants to maximize biological control of vineyard pests. *Journal of Applied Ecology* 43 (3), 547-554.

Behan-Pelletier, V.M. (1999) Oribatid mite biodiversity in agroecosystems: role for bioindication. *Agriculture Ecosystems & Environment* 74 (1-3), 411-423.

Benedict, J.H., Leigh, T.F. and Hyer, A.H. (1983) *Lygus hesperus* (Heteroptera: Miridae) Oviposition Behavior, Growth, and Survival in Relation to Cotton Trichome Density. *Environmental Entomology* 12: 331-335.

Bennison, J., Pope, T. and Maulden, K. (2010) The potential use of flowering alyssum as a 'banker' plant to support the establishment of *Orius laevigatus* in everbearer strawberry for biological control of western flower thrips. *IOBC-OILB Working group: Integrated Plant Protection in Fruit Crops Subgroup 'Soft Fruits'*

Berndt, L.A. and Wratten, S.D. (2005) Effects of alyssum flowers on the longevity, fecundity, and sex ratio of the leafroller parasitoid *Dolichogenidea tasmanica*. *Biological Control* 32 (1), 65-69.

Berndt, L.A., Wratten, S.D. and Scarratt, S.L. (2006) The influence of floral resource subsidies on parasitism rates of leafrollers (Lepidoptera : Tortricidae) in New Zealand vineyards. *Biological Control* 37 (1), 50-55.

Blaeser, P., Sengonca, C. and Zegula, T. (2004) The potential use of different predatory bug species in the biological control of *Frankliniella occidentalis* (Pergande) (Thysanoptera : Thripidae). *Journal of Pest Science* 77 (4), 211-219.

Bohac, J. (1999) Staphylinid beetles as bioindicators. *Agriculture Ecosystems & Environment* 74 (1-3), 357-372.

Bosco, L. and Tavella, L. (2008) Collection of *Orius* species in horticultural areas of northwestern Italy. *Bulletin of Insectology* 61 (1), 209-210.

Boyer, S. and Wratten, S.D. (2010) The potential of earthworms to restore ecosystem services after opencast mining - A review. *Basic and Applied Ecology* 11 (3), 196-203.

Braman, S.K. and Yeargan, K.V. (1989) Intraplant distribution of 3 *Nabis* species (Hemiptera: Nabidae) and impact of *Nabis roseipennis* on green cloverworm populations in soybean. *Environmental Entomology* 18 (2), 240-244.

Braman, S.K. and Yeargan, K.V. (1990) Phenology and Abundance of *Nabis americoferus*, *Nabis roseipennis* and *Nabis rufusculus* (Hemiptera: Nabidae) and Their Parasitoids in Alfalfa and Soybean. *Journal of Economic Entomology* 83 (3), 823-830.

Broufas, G.D. and Koveos, D.S. (2000) Effect of different pollens on development, survivorship and reproduction of *Euseius finlandicus* (Acari: Phytoseiidae). *Environmental Entomology* 29 (4), 743-749.

Brown, A.S.S., Simmonds, M.S.J. and Blaney, W.M. (1999) Influence of species of host plants on the predation of thrips by *Neoseiulus cucumeris*, *Iphiseius degenerans* and *Orius laevigatus*. *Entomologia Experimentalis et Applicata* 92 (3), 283-288.

Brown, M.W. and Tworkoski, T. (2004) Pest management benefits of compost mulch in apple orchards. *Agriculture Ecosystems & Environment* 103 (3), 465-472.

Buckerfield, J.C. and Webster, K.A. (1996) Earthworms, Mulching, Soil Moisture and Grape Yields. *The Australian and New Zealand Wine Industry Journal* 11 (1), 47-53.

Buckerfield, J.C. and Webster, K.A. (2002) Organic matter management in vineyards- mulches for soil maintenance. *The Australian and New Zealand Grapegrower and Winemaker* 461: 26-33.

Buckerfield, J.C. and Webster, K.A. (2001) Responses to mulch continue- results from five years of field trials. *The Australian and New Zealand Grapegrower and Winemaker* 453: 71-78.

Bugg, R.L. (1987) Observations on insects associated with a nectar-bearing chilean tree, *Quillaja-saponaria molina* (Rosaceae). *Pan-Pacific Entomologist* 63 (1), 60-64.

Burnip, G.M., Daly, J.M., Hackett, J.K. and Suckling, D.M. (2002) European earwig phenology and effect of understorey management on population estimation. *New Zealand Plant Protection* 55: 390-395.

Carvalho, L.M., Bueno, V.H.P. and Castañé, C. (2010) Evaluation of oviposition substrates for *Orius insidiosus* (Say) (Hemiptera, Anthocoridae). *Revista Brasileira de Entomologia* 54 (1), 115-119.

Castane, C., Alomar, O., Goula, M. and Gabarra, R. (2004) Colonization of tomato greenhouses by the predatory mired bugs *Macrolophus caliginosus* and Dicyphus tamaninii. *Biological Control* 30 591-597.

Clements, D.J. and Yeargan, K.V. (1997) Seasonal and intraplant distribution of *Orius insidiosus* (Hemiptera : Anthocoridae) on soybean and possible interactions with *Nabis roseipennis* (Hemiptera : Nabidae). *Journal of the Kansas Entomological Society* 70 (2), 94-99.

Cocuzza, G.E., De Clercq, P., Van de Veire, M., De Cock, A., Degheele, D. and Vacante, V. (1997) Reproduction of *Orius laevigatus* and *Orius albidipennis* on pollen and *Ephestia kuehniella* eggs. *Entomologia Experimentalis et Applicata* 82: 101-104.

Codron, J.M., Siriex, L. and Reardon, T. (2006) Social and environmental attributes of food products in an emerging mass market: Challenges of signaling and consumer perception, with European illustrations. *Agriculture and Human Values* 23 (3), 283-297.

Cohen, A.C. (1989) Ingestion efficiency and protein consumption by a Heteropteran predator. *Annals of the Entomological Society of America* 82 (4), 495-499.

Coll, M. (1996) Feeding and ovipositing on plants by an omnivorous insect predator. *Oecologia* 105 (2), 214-220.

Coll, M. (1998) Living and feeding on plants in predatory Heteroptera. In: Predatory Heteroptera: Their Ecology and Use in BiologicalControl. M. Coll, Ruberson, J.R. (Eds.), Lanham, MD, USA. pp. 89-130

Coll, M. and Bottrell, D.G. (1995) Predator-prey Association in Mono and Dicultures: Effect of Maize and Bean Vegetation. *Agriculture Ecosystems & Environment* 54 115-125.

Coll, M. and Guershon, M. (2002) Omnivory in terrestrial arthropods: Mixing plant and prey diets. *Annual Review of Entomology* 47: 267-297.

Coll, M. and Ridgway, R.L. (1995) Functional and numerical responses of *Orius insidiosus* (Heteroptera: Anthocoridae) to its prey in different vegetable crops. *Ecology and population biology* 88 (6), 732-738.

Colley, M.R. and Luna, J.M. (2000) Relative attractiveness of potential beneficial insectary plants to aphidophagous hoverflies (Diptera : Syrphidae). *Environmental Entomology* 29 (5), 1054-1059.

Corbett, A. and Rosenheim, J.A. (1996) Impact of natural enemy overwintering refuge and its interaction with the surrounding landscape. *Ecological Entomology* 21: 155-164.

Corey, D., Kambhampati, S. and Wilde, G. (1998) Electrophoretic analysis of *Orius insidiosus* (Hemiptera: Anthocoridae) feeding habits in field corn. *Journal of the Kansas Entomological Society* 71 (1), 11-17.

Cottrell, T.E. and Yeargan, K.V. (1998) Effect of pollen on *Coleomegilla maculata* (Coleoptera: Coccinellidae) population density, predation, and cannibalism in sweet corn. *Environmental Entomology* 27 (6), 1402-1410.

Coyle, D.R., Clark, K.E., Raffa, K.F. and Johnson, S.N. (2011) Prior host feeding experience influences ovipositional but not feeding preference in a polyphagous insect herbivore *Entomologia Experimentalis et Applicata* 138: 137-145.

Chase, M.W., Salamin, N., Wilkinson, M., Dunwell, J.M., Kesanakurth, R.P., Haidar, N. and Savolainen, V. (2005) Land plants and DNA barcodes: short-term and long-term goals. *Philosophical Transactions of the Royal Society, Series B* 360 1889-1895.

Chen, L. and Fadamiro, H.Y. (2006) Comparing the effects of five naturally occurring monosaccharide and oligosaccharide sugars on longevity and carbohydrate nutrient levels of a parasitic phorid fly, Pseudacteon tricuspis. *Physiological Entomology* 31 (1), 46-56.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y. and Leon, C. (2010) Validation of the ITS2 Region as a Novel DNA Barcode for Identifying Medicinal Plant Species. PLoSONE 5(1): e8613.

Chyzik, R., Klein, M. and Ben-Dov, Y. (1995) Reproduction and survival of the predatory bug *Orius albidipennis* on various arthropod prey. *Entomologia Experimentalis et Applicata* 75: 27-31.

Daily, G.C. (1997) Nature´s Services: Societal Dependence on Natural Ecosystems. *Island Press, Washington, D.C.*

De Puysseleyr, V., Hofte, M. and De Clercq, P. (2011) Ovipositing *Orius laevigatus* increase tomato resistance against *Frankliniella occidentalis* feeding by inducing the wound response. *Arthropod-Plant Interactions* 5 (1), 71-80.

Debach, P. (1974) Biological Control by Natural Enemies. Cambridge, UK

Eilenberg, J., Hajek, A. and Lomer, C. (2001) Suggestions for unifying the terminology in biological control. *BioControl* 46 (4), 387-400.

Elmore, C.L., Roncoroni, J., Wade, L. and Verdegaal, P. (1997) Mulch plus herbicides effectively control vineyard weeds. *California Agriculture* 51 (2), 14-17.

Eubanks, M.D. and Denno, R.F. (1999) The ecological consequences of variation in plants and prey for an omnivorous insect. *Ecology* 80 (4), 1253-1266.

Eubanks, M.D. and Styrsky, J.D. (2005) Effects of plant feeding on the performance of omnivorous ‘‘predators’’. In: Plant-Provided Food and Herbivore–Carnivore Interactions. F. L. Wäckers, P. C. J. van Rijn, and J. Bruin. Cambridge University Press

Evans, H.F. (1976) Effect of prey density and host plant characteristics on oviposition and fertility in *Anthocoris confusus* (Reuter). *Ecological Entomology* 1 (3), 157-161.

Faraji, F., Janssen, A. and Sabelis, M.W. (2002) Oviposition patterns in a predatory mite reduce the risk of egg predation caused by prey. *Ecological Entomology* 27 (6), 660-664.

Faraji, F., Janssen, A. and Sabelis, M.W. (2001) Predatory mites avoid ovipositing near counterattacking prey. *Experimental and Applied Acarology* 25 (8), 613-623.

Fiedler, A.K. and Landis, D.A. (2007a) Attractiveness of Michigan native plants to arthropod natural enemies and herbivores. *Environmental Entomology* 36 (4), 751-765.

Fiedler, A.K. and Landis, D.A. (2007b) Plant characteristics associated with natural enemy abundance at Michigan native plants. *Environmental Entomology* 36 (4), 878-886.

Fiedler, A.K., Landis, D.A. and Wratten, S.D. (2008) Maximizing ecosystem services from conservation biological control: The role of habitat management. *Biological Control* 45 (2), 254-271.

Fischer, S., Linder, C. and Freuler, J. (1992) Biology and utilization of *Orius majusculus* Reuter (Heteroptera, Anthocoridae) for the control of the thrips *Frankliniella occidentalis* Perg. and *Thrips tabaci* Lind., in greenhouses. *Revue Suisse de Viticulture, d'Arboriculture et d'Horticulture* 24 (2), 119-127.

Fitzgerald, J. (2006) Biological control of thrips and aphids in tunnel-grown strawberry in UK. *Bulletin OILB/SROP* 29 (9), 21-26.

Fox, T.B., Landis, D.A., Cardoso, F.F. and DiFonzo, C.D. (2004) Predators Suppress *Aphis glycines* Matsumura Population Growth in Soybean. *Environmental Entomology* 33 (3), 608-618.

Frank, S.D., Wratten, S.D., Sandhu, H.S. and Shrewsbury, P.M. (2007) Video analysis to determine how habitat strata affects predator diversity and predation of *Epiphyas postvittana* (Lepidoptera : Tortricidae) in a vineyard. *Biological Control* 41 (2), 230-236.

Funao, T. and Yoshiyasu, Y. (1995) Development and fecundity of *Orius sauteri* (Poppius) (Hemiptera: Anthocoridae) reared on *Aphis gossypii* Glover and corn pollen. *Japanese Journal of Applied Entomology and Zoology* 39 (1), 84-85.

Gabarra, R., Alomar, O., Castane, C., Goula, M. and Albajes, R. (2004) Movement of greenhouse whitefly and its predators between in- and outside of Mediterranean greenhouses. *Agriculture Ecosystems & Environment* 102 (3), 341-348.

Gariepy, T.D., Kuhlmann, U., Gillott, C. and Erlandson, M. (2007) Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of Arthropods. *Journal of Applied Entomology* 131 (4), 225-240.

Gerling, D., Alomar, O. and Arnó, J. (2001) Biological control of *Bemisia tabaci* using predators and parasitoids. *Crop Protection* 20 (9), 779-799.

Gillespie, D.R. and McGregor, R.R. (2000) The functions of plant feeding in the omnivorous predator *Dicyphus hesperus*: water places limits on predation. *Ecological Entomology* 25 (4), 380-386.

Goubault, M. and Hardy, I.C.W. (2007) Deuterium marking of chemical emissions: detectability and fitness consequences of a novel technique for insect behavioural studies. *Entomologia Experimentalis et Applicata* 125 285-296.

Goula, M. (1986) Contribución al estudio de los Hemipteros (Insecta, Heteroptera, Familia Miridae). *Tesis Doctoral. Departamento de Zoología. Facultad de Biología. Universidad de Barcelona.*

Goula, M., Espinosa, M. and Padrós, J. (1993) Aproximación cualitativa a la fauna de *Orius sp.* (Heteroptera, Anthocoridae) en el Maresme (Barcelona). *Phytoma España* 54: 44-49.

Graham, H.M. and Jackson, C.G. (1982) Distribution of Eggs and Parasites of *Lygus* spp (Hemiptera: Miridae), *Nabis* spp. (Hemiptera: Nabidae) and *Spissistilus festinus* (Say) (Homoptera: Membracidae) on Plant Stems. *Annals of the Entomological Society of America* 75: 56-60.

Graham, H.M. and Jackson, C.G. (1981) Ovipositional sites of the minute pirate bug in alfalfa stems (Heteroptera: Anthocoridae). *Southwestern Entomologist* 6 (3), 190-194.

Greenstone, M.H., Rowley, D.L., Weber, D.C., Payton, M.E. and Hawthorne, D.J. (2007) Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of the Colorado potato beetle. *Bulletin of Entomological Research* 97 (2), 201-209.

Griffin, M.L. and Yeargan, K.V. (2002) Factors potentially affecting oviposition site selection by the lady beetle *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Environmental Entomology* 31 (1), 112-119.

Groenteman, R., Guershon, M. and Coll, M. (2006) Effects of leaf nitrogen content on oviposition site selection, offspring performance, and intraspecific interactions in an omnivorous bug. *Ecological Entomology* 31 (2), 155-161.

Guppy, J.C. (1986) Bionomics of the Damsel Bug *Nabis americoferus* Carayon (Hemiptera: Nabidae), a Predator of the Alfalfa Blotch Leafminer (Diptera: Agromyzidae). *Canadian Entomologist* 118 (8), 745-751.

Gurr, G., Wratten, S. and Altieri, M.A. (2004) Ecological Engineering for Pest Management: Advances in Habitat Manipulation for Arthropods. *CSIRO Publishing* Collingwood, Victoria, Australia

Gurr, G., Wratten, S. and Barbosa, P. (2000) Success in conservation biological control of arthropods. In: Gurr GM, Wratten SD (Eds) Biological Control: measures of success. Kluwer, Dordrecht, pp 105-132.

Gurr, G.M. and Wratten, S.D. (1999) 'Integrated biological control': a proposal for enhancing success in biological control. *International Journal of Pest Management* 45 (2), 81-84.

Gurr, G.M., Wratten, S.D., Kehrli, P. and Scarratt, S. (2005) Cultural manipulations to enhance biological control in Australia and New Zealand: progress and prospects.

Gurr, G.M., Wratten, S.D. and Luna, J.M. (2003) Multi-function agricultural biodiversity: pest management and other benefits. *Basic and Applied Ecology* 4 (2), 107-116.

Hamel, C., Schellenberg, M.P., Hanson, K. and Wang, H. (2007) Evaluation of the "bait-lamina test" to assess soil microfauna feeding activity in mixed grassland. *Applied Soil Ecology* 36: 199-204.

Harwood, J.D., Desneux, N., Yoo, H.J.S., Rowley, D.L., Greenstone, M.H., Obrycki, J.J. and O'Neil, R.J. (2007) Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. *Molecular Ecology* 16: 4390-4400.

Harwood, J.D., Yoo, H.J.S., Greenstone, M.H., Rowley, D.L. and O'Neil, R.J. (2009) Differential impact of adults and nymphs of a generalist predator on an exotic invasive pest demonstrated by molecular gut-content analysis. *Biological Invasions* 11 (4), 895-903.

Heimpel, G.E., Lee, J.C., Wu, Z.S., Weiser, L., Wackers, F. and Jervis, M.A. (2004) Gut sugar analysis in field-caught parasitoids: adapting methods originally developed for biting flies. *International Journal of Pest Management* 50 (3), 193-198.

Heinz, K.M., Van Driesche, R.G. and Parrella, M.P.E. (2004) Biocontrol in protected culture. Ball Publishing, Batavia, IL.

Hogg, B.N., Bugg, R.L. and Daane, K.M. (2011) Attractiveness of common insectary and harvestable floral resources to beneficial insects. *Biological Control* 56 (1), 76-84.

Hollingsworth, M., Clark, A., Forrest, L., Richardson, J., Pennington, T., Long, D., Cowan, R., Chase, M., Gaudeul, M. and Hollingsworth, P. (2009) Selecting barcoding loci for plants: evaluation of seven candidate loci with species level sampling in three divergent groups of land plants. *Molecular Ecology Resources* 9 439-457.

Hoogendoorn, M. and Heimpel, G.E. (2001) PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. *Molecular Ecology* 10 (8), 2059-2067.

Hosseini, R., Schmidt, O. and Keller, M.A. (2008) Factors affecting detectability of prey DNA in the gut contents of invertebrate predators: a polymerase chain reaction-based method. *Entomologia Experimentalis et Applicata* 126 (3), 194-202.

Huang, N., Enkegaard, A., Osborne, L.S., Ramakers, P.M.J., Messelink, G.J., Pijnakker, J. and Murphy, G. (2011) The Banker Plant Method in Biological Control. *Critical Reviews in Plant Sciences* 30 (3), 259-278.

Hulshof, J., Ketoja, E. and Vanninen, I. (2003) Life history characteristics of *Frankliniella occidentalis* on cucumber leaves with and without supplemental food. *Entomologia Experimentalis et Applicata* 108 (1), 19-32.

Husseini, M., Schumann, K. and Sermann, H. (1993) Rearing immature feeding stage of *Orius majusculus* (Heteroptera: Anthocoridae) on the acarid mite *Tyrophagus putriscentiae* Schr as new alternative prey. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie* 116 (2), 113-117.

Idris, A.B. and Grafius, E. (1995) Wildflowers as nectar sources for *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Yponomeutidae). *Environmental Entomology* 24 (6), 1726-1735.

Irvin, N.A. and Hoddle, M.S. (2007) Evaluation of floral resources for enhancement of fitness of *Gonatocerus ashmeadi*, an egg parasitoid of the glassy-winged sharpshooter, *Homalodisca vitripennis*. *Biological Control* 40 (1), 80-88.

Isenhour, D.J. and Marston, N.L. (1981) Seasonal Cycles of *Orius insidiosus* (Hemiptera:Anthocoridae) in Missouri Soybeans. *Journal of the Kansas Entomological Society* 54 (1), 129-142.

Isenhour, D.J. and Yeargan, K.V. (1982) Oviposition sites of *Orius insidiosus* (Say) and *Nabis* spp. in Soybean (Hemiptera: Anthocoridae and Nabidae). *Journal of the Kansas Entomological Society* 55 (1), 65-72.

Jacometti, M.A., Wratten, S.D. and Walter, M. (2007a) Management of understorey to reduce the primary inoculum of *Botrytis cinerea*: Enhancing ecosystem services in vineyards. *Biological Control* 40 (1), 57-64.

Jacometti, M.A., Wratten, S.D. and Walter, M. (2007b) Understorey management increases grape quality, yield and resistance to *Botrytis cinerea*. *Agriculture Ecosystems & Environment* 122 (3), 349-356.

Jones, V.P., Hagler, J.R., Brunner, J.F., Baker, C.C. and Wilburn, T.D. (2006) An inexpensive immunomarking technique for studying movement patterns of naturally occurring insect populations. *Environmental Entomology* 35 (4), 827-836.

Jonsson, M., Wratten, S.D., Landis, D.A., Tompkins, J.-M.L. and Cullen, R. (2010) Habitat manipulation to mitigate the impacts of invasive arthropod pests. *Biological Invasions* 12 (9), 2933-2945.

Juen, A. and Traugott, M. (2007) Revealing species-specific trophic links in soil food webs: molecular identification of scarab predators. *Molecular Ecology* 16 (7), 1545-1557.

Jurado-Rivera, J.A., Vogler, A.P., Reid, C.A.M., Petitpierre, E. and Gómez-Zurita, J. (2009) DNA barcoding insect-host plant associations. *Proceedings of the royal society B* 276: 639-648.

Keasar, T., Ney-Nifle, M., Mangel, M. and Swezey, S. (2001) Early oviposition experience affects patch residence time in a foraging parasitoid. *Entomologia Experimentalis et Applicata* 98 (2), 123-132.

Kiman, Z.B. and Yeargan, K.V. (1985) Development and Reproduction of the Predator *Orius insidiosus* (Hemiptera: Anthocoridae) Reared on Diets of Selected Plant-Material and Arthropod Prey. *Annals of the Entomological Society of America* 78 (4), 464-467.

King, R.A., Read, D.S., Traugott, M. and Symondson, W.O.C. (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology* 17 (4), 947-963.

Kratz, W. (1998) The bait-lamina test - General aspects, applications and perspectives. *Environmental Science and Pollution Research* 5 (2), 94-96.

Kress, W.J., Wurdack, K.J., Zimmer, E.A., Weigt, L.A. and Janzen, D.H. (2005) Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* 102 (23), 8369-8374.

Landis, D.A., Wratten, S.D. and Gurr, G.M. (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45: 175-201.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., A., W., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.G. (2007) *Clustal W and Clustal X version 2.0. Bioinformatics* 23 2947-2948.

Lavandero, B., Wratten, S., Hagler, J. and Jervis, M. (2004) The need for effective marking and tracking techniques for monitoring the movements of insect predators and parasitoids. *International Journal of Pest Management* 50 (3), 147-151.

Lee, J.C. and Heimpel, G.E. (2008) Floral resources impact longevity and oviposition rate of a parasitoid in the field. *Journal of Animal Ecology* 77 (3), 565-572.

Lee, J.C., Heimpel, G.E. and Leibee, G.L. (2004) Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. *Entomologia Experimentalis et Applicata* 111 (3), 189-199.

Leon-Beck, M. and Coll, M. (2007) Plant and prey consumption cause a similar reductions in cannibalism by an omnivorous bug. *Journal of Insect Behavior* 20 (1), 67-76.

Lester, P.J., Thistlewood, H.M.A. and Harmsen, R. (2000) Some effects of pre-release host-plant on the biological control of *Panonychus ulmi* by the predatory mite *Amblyseius fallacis*. *Experimental & Applied Acarology* 24: 19-33.

Limburg, D.D. and Rosenheim, J.A. (2001) Extrafloral nectar consumption and its influence on survival and development of an omnivorous predator, larval *Chrysoperla plorabunda* (Neuroptera: Chrysopidae). *Environmental Entomology* 30 (3), 595-604.

Lövei, G.L., Sopp, P.I. and Sunderland, K.D. (1990) Digestion rate in relation to alternative feeding in three species of polyphagous predators. *Ecological Entomology* 15 293-300.

Loya-Ramirez, J.G., Garcia-Hernandez, J.L., Ellington, J.J. and Thompson, D.V. (2003) The impact of interplanting crops on the density predation of hemiptera predators. *Interciencia* 28 (7), 415-420.

Lucas, E. and Brodeur, J. (1999) Oviposition site selection by the predatory midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). *Environmental Entomology* 28 (4), 622-627.

Lundgren, J.G. (2009) Relationships of Natural Enemies and Non-prey Foods. Springer. 460 pages.

Lundgren, J.G. (2011) Reproductive ecology of predaceous Heteroptera *Biological Control* 59 (1), 37-52.

Lundgren, J.G. and Fergen, J.K. (2006) The oviposition behavior of the predator *Orius insidiosus*: acceptability and preference for different plants. *BioControl* 51: 217-227.

Lundgren, J.G., Fergen, J.K. and Riedell, W.E. (2008) The influence of plant anatomy on oviposition and reproductive success of the omnivorous bug *Orius insidiosus*. *Animal Behaviour* 75: 1495-1502.

Lundgren, J.G., Moser, S.E., Hellmich, R.L. and Seagraves, M.P. (2010) The effects of diet on herbivory by a predaceous lady beetle. *Biocontrol Science and Technology* 21 (1), 71-74.

Lundgren, J.G. and Seagraves, M.P. (2011) Physiological benefits of nectar feeding by a predatory beetle. *Biological Journal of the Linnean Society* 104 (3), 661-669.

Lundgren, J.G. and Weber, D.C. (2010) Changes in digestive rate of a predatory beetle over its larval stage: Implications for dietary breadth. *Journal of Insect Physiology* 56 431-437.

Lundgren, J.G., Wyckhuys, K.A.G. and Desneux, N. (2009) Population responses by *Orius insidiosus* to vegetational diversity. *BioControl* 54 (1), 135-142.

Maher, B.J. and Logan, D.P. (2007) European earwigs, *Forficula auricularia*, and predation of scale insects in organic and conventionally managed kiwifruit. *New Zealand Plant Protection* 60: 249-253.

Martinez-Cascales, J.I., Cenis, J.L., Cassis, G. and Sanchez, J.A. (2006) Species identity of *Macrolophus melanotoma* (Costa 1853) and *Macrolophus pygmaeus* (Rambur 1839) (Insecta: Heteroptera: Miridae) based on morphological and molecular data and bionomic implications. . *Insect Systematics and Evolution and Development* 37 385-404.

Matheson, C.D., Muller, G.C., Junnila, A., Vernon, K., Hausmann, A., Miller, M.A., Greenblatt, C. and Schlein, Y. (2007) A PCR method for detection of plant meals from the guts of insects. *Organisms Diversity & Evolution* 7: 294-303.

Mathews, C.R., Bottrell, D.G. and Brown, M.W. (2002) A comparison of conventional and alternative understory management practices for apple production: multi-trophic effects. *Applied Soil Ecology* 21 (3), 221-231.

Mathews, C.R., Bottrell, D.G. and Brown, M.W. (2004) Habitat manipulation of the apple orchard floor to increase ground-dwelling predators and predation of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). *Biological Control* 30 (2), 265-273.

McIntyre, B.D., Gold, C.S., Ssali, H. and Riha, S.J. (2003) Effects of mulch location on banana weevil, soil and plant nutrients, soil water and biomass in banana fields. *Biology and Fertility of Soils* 39 (2), 74-79.

Mendes, S.M., Bueno, V.H.P. and Carvalho, L.M. (2005) Adequabilidade de diferentes substratos à oviposiçao do predador *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). *Neotropical Entomology* 34 (3), 415-421.

Miller, M.A., Muller, G.C., Kravchenko, V.D., Junnila, A., Vernon, K.K., Matheson, C.D. and Hausmann, A. (2006) DNA-based identification of Lepidoptera larvae and plant meals from their gut contents. *Russian Entomological Journal* 15 (4), 427-432.

Montserrat, M., Albajes, R. and Castane, C. (2000) Functional response of four Heteropteran predators preying on greenhouse whitefly (Homoptera: Aleyrodidae) and western flower thrips (Thysanoptera: Thripidae). *Environmental Entomology* 29 (5), 1075-1082.

Montserrat, M., Bas, C., Magalhaes, S., Sabelis, M.W., de Roos, A.M. and Janssen, A. (2007) Predators induce egg retention in prey. *Oecologia* 150 (4), 699-705.

Moreno-Ripoll, R., Gabarra, R. and Agustí, N. (2009) Tracking predation of Macrolophus pygmaeus and Nesidiocoris tenuis (Heteroptera: Miridae) in tomato protected crops by molecular methods. *IOBC/WPRS Bulletin* 49 307-312.

Mundy, D.C. and Agnew, R.H. (2002) Effects of mulching with vineyard and winery waste on soil fungi and botrytis bunch rot in Marlborough vineyards. *New Zealand Plant Protection* 55: 135-138.

Nafziger, T.D., Jr. and Fadamiro, H.Y. (2011) Suitability of some farmscaping plants as nectar sources for the parasitoid wasp, *Microplitis croceipes* (Hymenoptera: Braconidae): effects on longevity and body nutrients. *Biological Control* 56 (3), 225-229.

Naranjo, S.E. and Gibson, R.L. (1996) Phytophagy in predaceous Heteroptera: Effects on life history and population dynamics. In: Zoophytophagous Heteroptera: Implications for Life History and Integrated Pest Management. O. Alomar, Wiedenmann, R.N. (Eds.), Lanham, MD, USA. pp. 57-93

Olson, D.M., Fadamiro, H., Lundgren, J.G. and Heimpel, G.E. (2000) Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiological Entomology* 25 (1), 17-26.

Patt, J.M., Hamilton, G.C. and Lashomb, J.H. (1997) Foraging success of parasitoid wasps on flowers: Interplay of insect morphology, floral architecture and searching behavior. *Entomologia Experimentalis et Applicata* 83 (1), 21-30.

Pease, C.G. and Zalom, F.G. (2010) Influence of non-crop plants on stink bug (Hemiptera: Pentatomidae) and natural enemy abundance in tomatoes. *Journal of Applied Entomology* 134 (8), 626-636.

Perdikis, D., Fantinou, A. and Lykouressis, D. (2011) Enhancing pest control in annual crops by conservation of predatory Heteroptera. *Biological Control* 59 (1), 13-21.

Petanidou, T. (2005) Sugars in Mediterranean floral nectars: An ecological and evolutionary approach. *Journal of Chemical Ecology* 31 (5), 1065-1088.

Pfannenstiel, R.S. and Yeargan, K.V. (1998) Ovipositional preference and distribution of eggs in selected field and vegetable crops by *Nabis roseipennis* (Hemiptera: Nabidae). *Journal of Entomological Science* 33 (1), 82-89.

Phoofolo, M.W., Obrycki, J.J. and Lewis, L.C. (2001) Quantitative assessment of biotic mortality factors of the European corn borer (Lepidoptera: Crambidae) in field corn. *Journal of Economic Entomology* 94 (3), 617-622.

Pickett, C.H. and Bugg, R.L.E. (1998) Enhancing biological control. University of California Press. San Diego.

Pico, F.X. and Retana, J. (2000) Temporal variation in the female components of reproductive success over the extended flowering season of a Mediterranean perennial herb. *Oikos* 89 (3), 485-492.

Portillo, N., Alomar, O. and Wackers, F. (2012) Nectarivory by the plant-tissue feeding predator *Macrolophus pigmaeus* Rambur (Heteroptera: Miridae): nutritional redundancy or nutritional benefit? *Journal of Insect Physiology. DOI 10.1016/j.jinsphys.2011.12.013*

Putra, N.S., Yasuda, H. and Sato, S. (2009) Oviposition preference of two hoverfly species in response to risk of intraguild predation. *Applied Entomology and Zoology* 44 (1), 29-36.

Richards, L.A. and Harper, A.M. (1978) Ovipositon by *Nabis alternatus* (Hemiptera: Nabidae) in alfalfa. *The Canadian Entomologist* 110: 1359-1362.

Richards, P.C. and Schmidt, J.M. (1996a) Effets de divers compléments alimentaires sur la survie et la reproduction d'*Orius insidiosus* (Say) (Hemiptera: Anthocoridae). *The canadian Entomologist* 128 (2), 171-176.

Richards, P.C. and Schmidt, J.M. (1996b) The suitability of some natural and artificial substrates as oviposition sites for the insidious flower bug, *Orius insidiosus*. *Entomologia Experimentalis et Applicata* 80 (2), 325-333.

Ripa, R., Rodriguez, F., Funderburk, J.E. and Espinoza, F. (2001) Predation of Frankliniella occidentalis by *Orius insidiosus* on plant hosts serving as sources of populations infesting fruit orchards. *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera. Rita Marullo, Laurence Mound* Reggio Calabria, Italy

Riudavets, J. (1995) Predators of *Frankliniella occidentalis* (Per.) and *Thrips tabaci* Lind.: a review. *Wageningen Agric. Univ. Pap.* 95: 43-87.

Riudavets, J. and Castane, C. (1998) Identification and evaluation of native predators of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in the Mediterranean. *Environmental Entomology* 27 (1), 86-93.

Robinson, K.A., Jonsson, M., Wratten, S.D., Wade, M.R. and Buckley, H.L. (2008) Implications of floral resources for predation by an omnivorous lacewing. *Basic and Applied Ecology* 9 (2), 172-181.

Rosenheim, J.A. (2005) Intraguild predation of *Orius tristicolor* by *Geocoris* spp. and the paradox of irruptive spider mite dynamics in California cotton. *Biological Control* 32 (1), 172-179.

Ruberson, J.R., Tauber, M.J. and Tauber, C.A. (1986) Plant Fedding by *Podisus maculiventris* (Heteroptera: Pentatomidae): Effect on Survival, Development and Preoviposition Period. *Environmental Entomology* 15: 894-897.

Ruppel, R.F. (1983) Cumulative insect-days as an index of crop protection. *Journal of Economic Entomology* 76 375-377.

Rutledge, C.E. and O'Neil, R.J. (2005) *Orius insidiosus* (Say) as a predator of the soybean aphid, *Aphis glycines* Matsumura. *Biological Control* 33 (1), 56-64.

Sanchez, J.A., Gillespie, D.R. and McGregor, R.R. (2004) Plant preference in relation to life history traits in the zoophytophagous predator *Dicyphus hesperus*. *Entomologia Experimentalis et Applicata* 112 (1), 7-19.

Sandhu, H.S., Wratten, S.D., Cullen, R. and Case, B. (2008) The future of farming: The value of ecosystem services in conventional and organic arable land. An experimental approach. *Ecological Economics* 64 (4), 835-848.

Sass, C., Little, D.P., Stevenson, D.W. and Specht, C.D. (2007) DNA Barcoding in the Cycadales: Testing the Potential of Proposed Barcoding Markers for Species Identification of Cycads. . *PLoSONE 2(11), e1154*

Scarratt, S.L., Wratten, S.D. and Shishehbor, P. (2008) Measuring parasitoid movement from floral resources in a vineyard. *Biological Control* 46 (2), 107-113.

Schellhorn, N.A. and Andow, D.A. (1999) Cannibalism and interspecific predation: Role of oviposition behavior. *Ecological Applications* 9 (2), 418-428.

Seagraves, M.P. (2009) Lady beetle oviposition behavior in response to the trophic environment. *Biological Control* 51 (2), 313-322.

Seagraves, M.P. and Lundgren, J.G. (2010) Oviposition response by *Orius insidiosus* (Hemiptera: Anthocoridae) to plant quality and prey availability. *Biological Control* 55 (3), 174-177.

Seagraves, M.P., Riedell, W.E. and Lundgren, J.G. (2011) Oviposition Preference for Water-Stressed Plants in *Orius insidiosus* (Hemiptera: Anthocoridae). *Journal of Insect Behavior* 24 (2), 132-143.

Seagraves, M.P. and Yeargan, K.V. (2009) Importance of predation by *Coleomegilla maculata* larvae in the natural control of the corn earworm in sweet corn. *Biocontrol Science and Technology* 19: 1067-1079.

Seagraves, M.P. and Yeargan, K.V. (2006) Selection and evaluation of a companion plant to indirectly augment densities of *Coleomegilla maculata* (Coleoptera: Coccinellidae) in sweet corn. *Environmental Entomology* 35 (5), 1334-1341.

Shapiro, J.P., Shirk, P.D., Kelley, K., Lewis, T.M. and Horton, D.R. (2010) Identity of Two Sympatric Species of Orius (Hemiptera: Heteroptera: Anthocoridae). *Journal of Insect Science* 10 189.

Sigsgaard, L. (2005) Oviposition preference of *Anthocoris nemoralis* and *A. nemorum* (Heteroptera: Anthocoridae) on pear leaves affected by leaf damage, honeydew and prey. *Biocontrol Science and Technology* 15 (2), 139-151.

Sigsgaard, L. (2004) Oviposition preference of *Anthocoris nemorum* and *A. nemoralis* for apple and pear. *Entomologia Experimentalis et Applicata* 111 (3), 215-223.

Silberbauer, L., Yee, M., Del Socorro, A., Wratten, S., Gregg, P. and Bowie, M. (2004) Pollen grains as markers to track the movements of generalist predatory insects in agroecosystems. *International Journal of Pest Management* 50 165-171.

Silveira, L.C.P., Bueno, V.H.P., Louzada, J.N.C. and Carvalho, L.M. (2005) Species of Orius (Hemiptera: Anthocoridae) and thrips (Thysanoptera): interaction in the same habitat? *Revista Arvore* 29 (5), 767-773.

Sinia, A., Roitberg, B., McGregor, R.R. and Gillespie, D.R. (2004) Prey feeding increases water stress in the omnivorous predator *Dicyphus hesperus*. *Entomologia Experimentalis et Applicata* 110 (3), 243-248.

Skirvin, D.J., Kravar-Garde, L., Reynolds, K., Jones, J., Mead, A. and Fenlon, J. (2007) Supplemental food affects thrips predation and movement of *Orius laevigatus* (Hemiptera: Anthocoridae) and *Neoseiulus cucumeris* (Acari: Phytoseiidae). *Bulletin of Entomological Research* 97 (3), 309-315.

Sloderbeck, P.E. and Yeargan, K.V. (1983) Comparison of *Nabis americoferus* and *Nabis roseipennis* (Hemiptera: Nabidae) as Predators of the Green Cloverworm (Lepidoptera: Noctuidae). *Environmental Entomology* 12 (1), 161-165.

Spellman, B., Brown, M.W. and Mathews, C.R. (2006) Effect of floral and extrafloral resources on predation of *Aphis spiraecola* by *Harmonia axyridis* on apple. *BioControl* 51 (6), 715-724.

Stang, M., Klinkhamer, P.G.L. and van der Meijden, E. (2006) Size constraints and flower abundance determine the number of interactions in a plant-flower visitor web. *Oikos* 112 (1), 111-121.

Staudacher, K., Wallinger, C., Schallhart, N. and Traugott, M. (2011) Detecting ingested plant DNA in soil-living insect larvae. *Soil Biology & Biochemistry* 43 (2), 346-350.

Stephens, A.E.A., Barrington, A.M., Bush, V.A., Fletcher, N.M., Mitchell, V.J. and Suckling, D.M. (2008) Evaluation of dyes for marking painted apple moths (*Teia anartoides* Walker, Lep. Lymantriidae) used in a sterile insect release program. *Australian Journal of Entomology* 47 131-136.

Stoner, A. (1972) Plant feeding by *Nabis*, a predaceus genus. *Environmental Entomology* 1 (5), 557-558.

Suckling, D.M., Burnip, G.M., Hackett, J. and Daly, J.C. (2006) Frass sampling and baiting indicate European earwig (*Forficula auricularia*) foraging in orchards. *Journal of Applied Entomology* 130 (5), 263-267.

Taylor, D.H., Williams, C.F. and Nelson, S.D. (1991) Measuring water infiltration rates of sports turf areas. *Agronomy Journal* 83 (2), 427-429.

Taylor, R.M. and Pfannenstiel, R.S. (2009) How Dietary Plant Nectar Affects the Survival, Growth, and Fecundity of a Cursorial Spider *Cheiracanthium inclusum* (Araneae: Miturgidae). *Environmental Entomology* 38 (5), 1379-1386.

Thomson, L.J. and Hoffmann, A.A. (2007) Effects of ground cover (straw and compost) on the abundance of natural enemies and soil macro invertebrates in vineyards. *Agricultural and Forest Entomology* 9 (3), 173-179.

Thomson, L.J., Sharley, D.J. and Hoffmann, A.A. (2007) Beneficial organisms as bioindicators for environmental sustainability in the grape industry in Australia. *Australian Journal of Experimental Agriculture* 47 (4), 404-411.

Tommasini, M.G., Van Lenteren, J.C. and Burgio, G. (2004) Biological traits and predation capacity of four *Orius* species on two prey species. *Bulletin of Insectology* 57 (2), 79-93.

Trottin-Caudal, Y., Grasselly, D., Trapateau, M., Dobelin, H. and Millot, P. (1991) Lutte biologique contre *Frankliniella occidentalis* avec *Orius majusculus* sur concombre. *Bulletin SROP* 14 (5), 50-56.

Tscharntke, T., Bommarco, R., Clough, Y., Crist, T.O., Kleijn, D., Rand, T.A., Tylianakis, J.M., van Nouhuys, S. and Vidal, S. (2007) Conservation biological control and enemy diversity on a landscape scale. *Biological Control* 43 (3), 294-309.

Vacante, V., Cocuzza, G.E., De Clercq, P., Van de Veire, M. and Tirry, L. (1997) Development and survival of *Orius albidipennis* and *Orius laevigatus* (Heteroptera: Anthocoridae) on various diets. *Entomophaga* 42 (4), 493-498.

Valentini, A., Miquel, C., Nawaz, M.A., Bellemain, E., Coissac, E., Pompanon, F., Gielly, L., Cruaud, C., Nascetti, G., Wincker, P., Swenson, J.E. and Taberlet, P. (2009) New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. *Molecular Ecology Resources* 9 (1), 51-60.

Van Handel, E. (1985a) Rapid determination of glycogen and sugars in mosquitos. *Journal of the American Mosquito Control Association* 1 (3), 299-301.

Van Handel, E. (1985b) Rapid determination of total lipids in mosquitos. *Journal of the American Mosquito Control Association* 1 (3), 302-304.

Vattala, H.D., Wratten, S.D., Phillips, C.B. and Wackers, F.L. (2006) The influence of flower morphology and nectar quality on the longevity of a parasitoid biological control agent. *Biological Control* 39 (2), 179-185.

Wäckers, F. (2005) Plant-provided food for carnivorous insects: a protective mutualism and its applications. Cambridge: Cambridge University Press.

Wäckers, F.L. (2001) A comparison of nectar and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *Journal of Insect Physiology* 47 (9), 1077-1084.

Wanner, H., Gu, H., Günther, D., Hein, S. and Dorn, S. (2006) Tracing spatial distribution of parasitism in fields with flowering plant strips using stable isotope marking. *Biological Control* 39 240-247.

Weber, D.C. and Lundgren, J.G. (2009) Detection of predation using qPCR: Effect of prey quantity, elapsed time, chaser diet, and sample preservation on detectable quantity of prey DNA. *Journal of Insect Science* 9. Article 41.

Winkler, K., Wackers, F. and Pinto, D.M. (2009) Nectar-providing plants enhance the energetic state of herbivores as well as their parasitoids under field conditions. *Ecological Entomology* 34 (2), 221-227.

Winkler, K., Wackers, F., Termorshuizen, A.J. and van Lenteren, J.C. (2010) Assessing risks and benefits of floral supplements in conservation biological control. *BioControl* 55: 719-727.

Yokoyama, V.Y. (1978) Relation of seasonal changes in extrafloral nectar and foliar protein and arthropod populations in cotton. *Environmental Entomology* 7 (6), 799-802.

Zaki, F.N. (1989) Rearing of 2 predators, *Orius albidipennis* (Reuter) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) on some insect larvae. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie* 107 (1), 107-109.