Control biológico de la psila del peral: estudios de sus enemigos naturales y aspectos de su comunicación química

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Tesis aprobada por el tribunal integrado por Ing. Agr. (Dra.) Stella Zerbino, Lic. Biol. (Dra.) Liliana Cichon y Lic. Biol. (Dra.) Patricia Carina Fernández el mes de abril de 2023. Autora: dottoressa in scienze e tecnologie Agrarie, MSC, Diana Valle López. Director: B. Q. PhD. Andrés González. Codirectora: Ing. Agr. Dra. María Valentina Mujica

A mis padres.
A Pablo y Tommy.
A Marcelo y Mariana,
siempre en el corazón.

AGRADECIMIENTOS

Un agradecimiento muy especial al equipo de protección vegetal de INIA Las Brujas, Mariana Silvera, Yesica Bernaschina, Lucia Goncálvez, Wilma Walasek, Gonzalo Vázquez, Gastón Tejera, Peter Schlenzak, Jonathan Macchi, Jorge Secco, y Alfredo Fernández por todo el soporte. A Carolina Leoni, Diego Maeso y Roberto Zoppolo por haber incentivado el desarrollo de esta tesis y por los invalorables consejos y aportes durante todo el proceso.

A los LEQUERS, nuevos y viejos que con sus aportes enriquecieron este trabajo. Un agradecimiento muy especial a Hernan Groba, Paula Lagurara, Anna Burgueño, Maria Eugenia Amorós, Federico Rodrigo, Guillermo Rehermann y Maria Laura Umpierrez, por los intercambios de ideas y consejos, y por haberse convertidos en amigos inigualables. A Carmen Rossini por haberme hecho crecer en todo este recorrido académico.

A Maria Inés Siri, Felipe Clavijo, Nuri Cabrera y Blas Lavandero que abrieron sus laboratorios para que pudiera formarme en las técnicas moleculares. Enrique Morelli y Blas Lavandero, integrantes del comité de seguimiento, que con sus aportes hicieron este trabajo más completo.

A mis tutores, Andrés Gonzalez y Valentina Mujica, que creyeron en este proyecto y me permitieron desarrollarlo en total libertad, aportando y acompañado cada etapa.

A los productores que nos prestaron sus quintas para los muestreos, siempre mostrando buena disposición y total disponibilidad para acceder a los predios.

A INIA, por haberme apoyado con la beca que financió este Doctorado, y por haberme hecho parte de esta institución.

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RESUMEN

La psila del peral, *Cacopsylla bidens* (Hemiptera, Psyllidae), es una de las plagas que ha adquirido un rol primario en el cultivo de peral. El control de este hemíptero se basa en el uso de productos de síntesis, por lo que se hace necesario buscar estrategias alternativas de control. Para responder a esta demanda, este trabajo tuvo como objetivo profundizar los conocimientos sobre las redes tróficas, la biología y la comunicación química del gremio de los predadores, para poder ajustar un plan de control biológico de este insecto. Se determinó mediante técnicas moleculares cuál de los predadores presentes en los montes se estaba alimentando de *C. bidens*. Asimismo, se determinó en qué momento de la temporada estos enemigos naturales empiezan a actuar. Los resultados revelaron que hay diferentes predadores que se alimentan de la plaga, pertenecientes a los órdenes Coleoptera y Neuroptera. En particular, *Chrysoperla externa* fue el predador que con mayor frecuencia se alimentó de *C. bidens.* A su vez, se estableció que estos predadores empiezan a actuar de forma más relevante recién sobre la tercera generación de *C. bidens*. Con el fin de poder atraer y así anticipar la llegada de *C. externa* en la temporada, se caracterizó el perfil de volátiles emitidos por la planta de peral luego de ser dañada por *C. bidens*. También, a modo comparativo, se caracterizaron los volátiles emitidos por herbivoría luego del daño por el lepidóptero *Argyrotaenia sphaleropa*. Los resultados demostraron que la planta de peral responde a la herbivoría modificando su perfil de volátiles, siendo este cambio específico según el herbívoro. Por último, se evaluó si esta variación en el perfil de volátiles podría estimular una respuesta en el predador *C. externa*. Se observó que éste se beneficia del cambio químico, discriminando entre las plantas sanas y dañadas por ambos herbívoros, siendo más atraídos por los volátiles emitidos cuando la planta es dañada por *C. bidens.* Estos resultados aportan información clave para poder transitar hacia un manejo más sustentable de la plaga.

Palabras clave: *Cacopsylla bidens*, *Chrysoperla externa*, *Argyrotaenia sphaleropa,* ecología molecular, VOC, HIPV.

Biological control of the pear psylla: studies of its natural enemies and aspects of its chemical communication

SUMMARY

The pear psylla*, Cacopsylla bidens* (Hemiptera, Psyllidae), is one of the pests that has acquired a primary role in pear cultivation. The management of the pear psylla relies on the use of chemical control, so finding alternate control methods is warranted. To meet this demand, this project seeks to improve the understanding of the biology, chemical communication and trophic webs of the predator guild in order to design a biological control strategy. Molecular tools were used to determine which of the predators present in the pear orchard was feeding on *C. bidens*. The findings revealed that many predators from the orders Coleoptera and Neuroptera feed on the pest, with *Chrysoperla externa* as the predator that most frequently fed on *C. bidens*. The dynamics of predator presence during the season was also studied. It was determined that these predators are present in relevant numbers only during the third generation of *C. bidens*. Seeking to attract and potentially anticipate the arrival of *C. externa* in the season, changes in the volatile profile of pear plants as a result of psylla damage were investigated. For comparison, volatile changes induced by *Argyrotaenia sphaleropa* leafroller larvae were also identified. The results showed that the pear tree modifies its volatile profile because of herbivore damage, and that this alteration is specific to the herbivore. The predator *C. externa* was in turn evaluated to determine whether this chemical change may trigger a behavioral response, finding that the predator discriminates between undamaged plants and plants damaged by both herbivores, showing more attraction to volatiles released by plants damaged by *C. bidens*. These findings provide relevant information that may be used to move towards a more sustainable management of the pest.

Keywords: *Cacopsylla bidens*, *Chrysoperla externa, Argyrotaenia sphaleropa*, molecular ecology, VOCs, HIPVs

1. INTRODUCCIÓN

El aumento de la demanda mundial de alimentos, conjuntamente con la necesidad de reducir el impacto ambiental de las prácticas agrícolas, requiere una intensificación sustentable de la producción (Bommarco et al., 2013,Cerutti et al., 2011, Godfray et al., 2010). En la actualidad, la producción agrícola se encuentra subordinada al uso de insumos externos, resultando en una marcada dependencia de la producción a estas herramientas (Godfray et al., 2010, Granatstein y Kupferman, 2006). En el caso concreto de la fruticultura moderna, este hecho se hace aún más evidente siendo que, además de un aumento productivo, se requiere cumplir con ciertos estándares de calidad estéticos demandados por los consumidores (Simon et al., 2011). Por estos motivos, las plagas y enfermedades han sido históricamente una limitante de la producción, y su control se ha basado principalmente en el control químico, que a lo largo de las décadas ha venido causando una serie de efectos negativos tanto sobre el medioambiente como sobre la salud humana (Nicolopoulou-Stamati et al., 2016, Sánchez-Bayo y Wyckhuys, 2019).

Con el fin de poder obtener un agricultura sustentable, la producción debería poder tomar ventaja de las relaciones biológicas que ocurren naturalmente en un sistema, buscando incrementar y favorecer estas interacciones entre los diferentes integrantes del ecosistema, con el fin de maximizar los servicios ecosistémicos (Granatstein y Kupferman, 2006). Es en este marco que las tecnologías que se basan en los principios de la ecología adquieren un rol primario, con el fin de obtener una producción que se pueda sustentar en el largo plazo (German et al., 2017, Granatstein y Peck, 2017).

Con el foco en esta necesidad, en el ámbito nacional, se ha buscado controlar las dos principales plagas frutícolas, *Cydia pomonella* y *Grapholita molesta* (Lepidoptera:

Tortricidae)*,* hacia las cuales estuvieron dirigidas históricamente la mayoría de las aplicaciones de insecticidas en la fruticultura (Núñez y Scatoni, 2013). Durante la última década se ha ido desarrollando el denominado Programa de Manejo Regional de Plagas, el cual basándose en la herramienta de la confusión sexual mediada por feromonas, ha permitido una reducción significativa en las aplicaciones dirigidas a estas, aunque manteniendo la necesidad de aplicaciones sobre las plagas secundarias (Zoppolo et al., 2016).

Entre las secundarias, la psila del peral *Cacopsylla bidens* (Hemiptera, Psyllidae) ha adquirido protagonismo tanto por los daños directos causados por la alimentación de adultos y ninfas, como por ser el vector candidato de un fitoplasma, *Candidatus fitoplasma pyri* (Seemüller y Schneider, 2004). El control de esta plaga se ha basado históricamente en el uso de insecticidas y se ha aconsejado orientar las aplicaciones sobre las poblaciones invernantes (DIGEGRA, 2018, Escudero-Colomar et al., 2021). La aparición de resistencia a los principales principios activos autorizados, conjuntamente al marcado desequilibrio ecológico al cual se enfrenta el sistema, hace que las estrategias actuales fallen en controlar la plaga, lo que vuelve necesarias varias aplicaciones durante la temporada, especialmente en postcosecha, momento en el cual se registran las poblaciones más abundantes de *C. bidens* (Civolani, 2012).

Enmarcado en la necesidad de encontrar estrategias alternativas al control de esta plaga, en el ámbito nacional se llevó a cabo un estudio dirigido a promover una estrategia de control biológico de conservación. Es así que se registraron los principales enemigos naturales presentes en los montes de peral, asociados a la presencia de *C. bidens*, y se determinó que existe en el país un ensamble de predadores muy variados que podrían influir en las dinámicas poblacionales de la plaga (Valle et al., 2021). A su vez, se observó la presencia de un parasitoide de ninfas perteneciente a la familia *Encyritdae*, pero sus capturas fueron extremadamente bajas, especialmente en los momentos de mayor abundancia de ninfas, lo que hace suponer que este microhimenóptero no sería el mejor candidato para un plan de control biológico (Valle et al., 2021).

Luego de esta investigación preliminar, se encontró pertinente continuar y profundizar los estudios inherentes a la ecología de este gremio de predadores, especialmente considerando que se caracterizan por sus hábitos generalistas a la hora de alimentarse. Los resultados obtenidos indicaron que uno de los aspectos que podrían explicar la ineficiencia en controlar la plaga podría radicarse en la asincronía entre la llegada de los predadores a los montes de peral y el ciclo de la plaga (Heimpel y Asplen, 2011, Heimpel y Mills, 2017, Mills y Heimpel, 2018).

Debido a estos antecedentes, esta tesis de doctorado trata de elucidar, mediante técnicas moleculares, las redes tróficas de los predadores asociados a la presencia de *C. bidens*, con el fin de determinar cuál de las especies podrían ser buenos candidatos para un plan de control biológico. A su vez, se buscó profundizar en las claves químicas que podrían mediar el encuentro entre presa y predador, siendo esta una herramienta que podría ser utilizada para favorecer la presencia y permanencia de este gremio en los montes de interés.

2. MARCO TEÓRICO

2.1. FRUTICULTURA EN URUGUAY

La producción frutícola en Uruguay se ubica principalmente en la zona periurbana de Montevideo, cubriendo un total de 4,5 miles de hectáreas con 611 productores en la actualidad. Los predios son generalmente pequeños, con un promedio de 7 hectáreas, con conducción familiar. Los principales productos, manzanas, peras y durazno, están destinados casi en la totalidad al consumo interno, y en menor escala a la exportación, representando apenas el 1 % del total de la fruta fresca exportada (MGAP-DIEA, 2021).

Este sector se ve afectado por numerosas plagas y enfermedades; en el ámbito nacional, las principales plagas pertenecen al orden Lepidoptera y Hemiptera (Núñez y Scatoni, 2013). Para poder obtener fruta que cumpla con los estándares cualitativos y cuantitativos requeridos para su comercialización, se hace necesario optar por medidas de control centralizadas en el uso de plaguicidas de síntesis (Simon et al., 2011). Estos productos inciden en los costos de producción de forma significativa, representando el 15 % de los costos totales y ubicándose únicamente por debajo de los costos de mano de obra (MGAP-Opypa, 2017). Además de ser un insumo de alto costo para los productores, los plaguicidas de síntesis representan un riesgo para el medioambiente debido a los residuos en aguas, suelos y partes vegetales que se puedan generar, siendo nocivos tanto para el aplicador como para los consumidores. Conjuntamente, el uso de estos plaguicidas ha llevado a un marcado desequilibrio ecológico, con la desaparición de varios enemigos naturales y el declive de los polinizadores (Calvo-Agudo et al., 2019, Nicolopoulou-Stamati et al., 2016, Sánchez-Bayo y Wyckhuys, 2019). El conjunto de estos efectos negativos nos traslada a la etapa actual, donde se hace más evidente la necesidad de encontrar estrategias alternativas al uso intensivo de plaguicidas, para poder generar una producción que sea sostenible y sustentable en el largo plazo, en línea con un plan agroecológico del cultivo.

2.2. LA PSILA DEL PERAL

2.2.1. Descripción y bioecología

Cacopsylla bidens, comúnmente conocida como psila del peral, es un pequeño hemíptero esternorrinco que se caracteriza por ser monofago con alto grado de especificidad sobre el hospedero (Burckhardt, 1994a, b, Moharum, 2013).

Las especies pertenecientes al género *Cacopsylla* son generalmente de hábitos diurnos, y los adultos tienen alas anteriores bien desarrolladas y el último par de patas adaptado para el salto, por lo cual suelen poder desplazarse activamente en el cultivo (Burckhardt, 1994a, Burckhardt y Hodkinson, 1986). Las alas anteriores presentan una singular venación, y en el caso específico de *C. bidens* se destaca una mancha característica en la parte inferior, que se hace notoria cuando el adulto se encuentra en reposo (Claps et al., 2008). La diferenciación de esta especie respecto a las demás del mismo género se basa en los caracteres del macho adulto, ya que *C. bidens* presenta parámeros con un proceso apical dentado y la porción apical del aedeago más redondeada (Burckhardt y Hodkinson, 1986, Valle et al., 2017). *Cacopsylla bidens*, como la mayoría de las especies que conforman este género, se caracteriza por tener un marcado dimorfismo dictado por el fotoperíodo, el morfotipo de verano es de coloración clara, generalmente entre el amarillo y el verde, y de menor tamaño respecto al morfotipo de invierno, cuyas características son la coloración oscura y el tamaño mayor (Soroker et al., 2013, Soroker et al., 2003). Este último es la forma en que los adultos transcurren el invierno, en diapausa reproductiva, mientras que el morfotipo de verano es la forma reproductiva de la especie (Hodkinson, 2009, Madar et al., 2017) (fig. 1.).

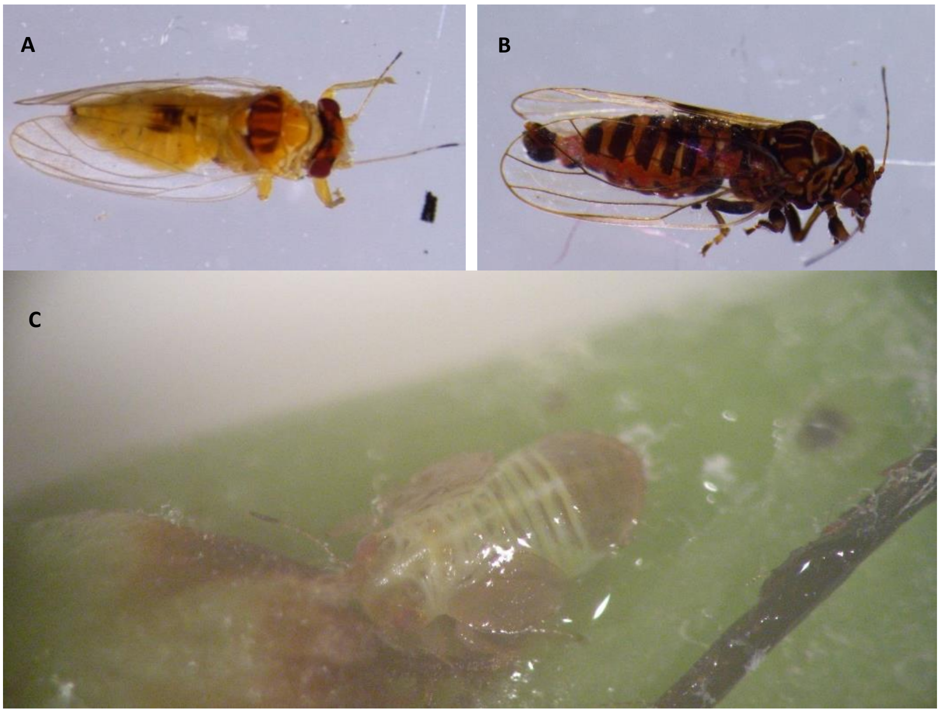

Fig 1. Adultos de *Cacopsylla bidens* en sus morfotipos de verano (A) y de invierno (B). Ninfa de quinto estadio de *Cacopsylla bidens* alimentándose (C)

El ciclo de este hemíptero en nuestras condiciones climáticas comienza en el mes de agosto, cuando las hembras invernantes cesan la diapausa reproductiva y empiezan las posturas que darán origen a la primera generación de ninfas de la temporada (Núñez y Scatoni, 2013, Valle et al., 2017, Valle et al., 2021). Luego de pasar por 5 estadios ninfales emergen los adultos. Las ninfas son generalmente gregarias en los primeros estadios y luego se empiezan a desplazar por las hojas y las ramas hasta llegar al último estadio, que se caracteriza por presentar esbozos alares bien definidos (Burckhardt y Hodkinson, 1986). Para completar una generación, se necesita la acumulación de 366 grados día, y en nuestras condiciones pueden cumplir entre 7 y 8 generaciones por año, dependiendo de las condiciones climáticas (Mujica et al., 2014, Shaltiel-Harpaz et al., 2014). Usualmente, los picos de mayor población tanto de adultos como de ninfas, en nuestras condiciones, se registran en la época de postcosecha, desde febrero hasta mayo (Valle et al., 2021).

2.2.2. Daños

La psila del peral es considerada una plaga clave del cultivo en los diferentes países en los que está registrada su presencia. Los daños causados por este hemíptero se pueden agrupar en dos tipos: directos e indirectos (Burckhardt, 1994a, b).

Entre los daños de tipo directo, el principal es debido a la alimentación sobre los tejidos verdes, tanto de adultos como de los estadios inmaduros, por la remoción de savia y consecuente disminución de la actividad fotosintética. Conjuntamente a la remoción de savia, los adultos inyectan una toxina mediante la saliva, la cual interfiere con la translocación de nutrientes en la planta causando, en elevadas poblaciones, que las plantas expresen un complejo de síntomas conocido como *Psylla shock*. Entre los síntomas atribuidos a esta toxina se destaca la caída prematura de las hojas, la reducción en el crecimiento de la planta, la disminución en el tamaño de las frutas, y afectaciones sobre la maduración de éstas en el año siguiente (Alston y Murray, 2007, Westigard y Zwick, 1972).

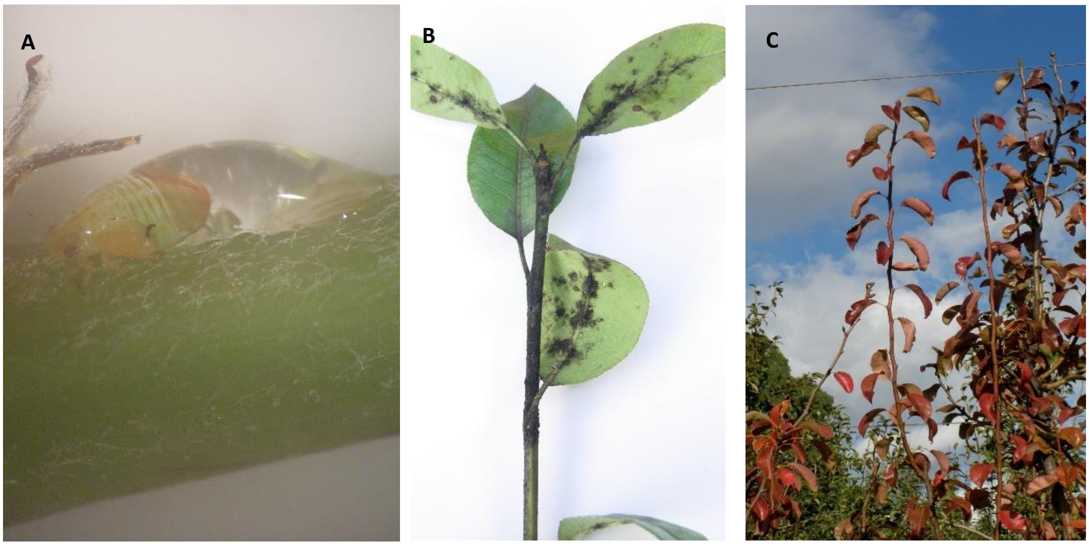

Fig. 2. Principales daños. (A) Ninfa de *Cacopsylla bidens* excretando mielecilla, (B) aparición de fumagina (foto: V. Mujica) y (C) síntomas de *Pear Decline* campo (foto: D. Maeso).

Asimismo, las ninfas durante el proceso de alimentación excretan una sustancia azucarada conocida como mielecilla, que es el substrato ideal para la proliferación de un conjunto de hongos cuyo síntoma característico es la aparición de fumagina, la cual coloniza los ramilletes y las hojas, debilita la planta y causa daños estéticos a la fruta, sobre todo en el continente europeo (Horton, 1999). En nuestras condiciones, debido a que los picos poblacionales de la plaga se dan en postcosecha, los daños en la fruta suelen ser insignificantes (Núñez y Scatoni, 2013).

Entre los daños indirectos, las especies pertenecientes al género *Cacopsylla* son vectores de un fitoplasma, *Candidatus phytoplasma pyri*, cuyos síntomas abarcan el enrojecimiento temprano de los árboles afectados, el marchitamiento de las raíces con consecuente inanición de la planta, el menor número de brotes y el aborto de yemas, incluyendo en los casos más graves la muerte del cultivo (Fernández y Conci, 2019, Seemüller y Schneider, 2004). Esta enfermedad ha sido reportada en Uruguay, con presencia del subgrupo 16SrX-C, asociado a la presencia de *C. bidens* (Maeso, 2020).

2.2.3. Estrategias de control

En la actualidad, las estrategias de control que mayoritariamente se buscan promover son acordes a los lineamientos del manejo integrado de plagas y enfermedades, por lo cual se hace necesario tomar en cuenta los factores varietales y de manejo de cultivo que han demostrado tener un efecto directo sobre las poblaciones de la plaga y la tolerancia a los ataques por parte del peral (Civolani, 2012, Maeso, 2020, Weddle et al., 2009). La fertilización nitrogenada excesiva favorece la presencia de hemípteros sobre los cultivos, tanto por efecto directo, actuando sobre la reducción de algunos compuestos que afectan negativamente a los herbívoros, como por efecto indirecto, por aumento de la vegetación que permite un mejor desarrollo de las plagas (Civolani, 2012, Shaltiel-Harpaz et al., 2014, Weddle et al., 2009). Los lineamientos productivos indican la posibilidad de usar productos de síntesis, orientados principalmente al control de las poblaciones invernantes, optando generalmente por el uso de aceites minerales (solos o en mezcla), si bien en la mayoría de los casos se hacen necesarias más aplicaciones durante la temporada (Civolani, 2012, DIGEGRA, 2018).

El conjunto de herramientas para el manejo de esta plaga disponibles actualmente es limitado e ineficiente en el largo plazo para mantener las poblaciones controladas. Esta situación se observó en varios países europeos, en los que desde los años 60 los psílidos han adquirido mayor relevancia como plagas primarias de los perales. Esto se debe a que la eficiencia de control de los diferentes productos de síntesis declinó, principalmente por la selección de poblaciones resistentes, conjuntamente a los efectos negativos sobre la fauna benéfica entendida como los controladores naturales de psila (Buès et al., 2003, Civolani, 2012, Civolani et al., 2010, Esmaeily et al., 2022).

Como consecuencia de esta situación, en la actualidad es necesario encontrar estrategias alternativas y un cambio de paradigma, tanto para el control de esta plaga como para el manejo general de los sistemas agropecuarios.

2.3. CONTROL BIOLÓGICO

El cambio de paradigma productivo que condujo a la limitación y/o eliminación de algunos principios activos, conjuntamente con el éxito obtenido por el Programa de Manejo Regional de Plagas en la reducción de aplicaciones de insecticidas dirigidos a los principales lepidópteros plagas, permite buscar estrategias alternativas al control de plagas (Amarasekare y Shearer, 2017, Zoppolo et al., 2016).

El control biológico, adquiere un rol primario en la regulación de las poblaciones de las plagas en los cultivos, en la óptica de una producción sustentable (Wyckhuys et al., 2013).

2.3.1. Definición y tipos de control biológico

Se define el control biológico como la supresión de las poblaciones de un organismo, considerado nocivo o perjudicial para el ser humano, por medio de organismos vivos o virus. Este tipo de control se basa en el efecto positivo indirecto de los agentes de control biológico sobre las actividades humanas, mediada por efectos negativos directos o indirectos de estos agentes sobre las poblaciones de una o más especies blanco (Heimpel y Mills, 2017).

El control biológico se puede clasificar en cuatro categorías —dos de las cuales suponen la intervención directa de la aplicación de organismos adicionales a los naturalmente presentes— y se diferencia entre el control biológico clásico, que prevé la introducción de controladores exóticos, y el control biológico aumentativo, cuyo fin es liberar periódicamente enemigos naturales provenientes de crías masivas para generar una población estable de controladores. A las otras dos categorías pertenecen el control biológico natural y el de conservación, que suponen la acción de organismos biocontroladores naturalmente presentes en el ecosistema (Eilenberg et al., 2001, Heimpel y Mills, 2017, Stenberg et al., 2021). El control biológico de conservación tiene como objetivo favorecer la presencia de la entomofauna benéfica brindando un hábitat favorable y disminuyendo el impacto de los insecticidas sobre la mortalidad de los enemigos naturales, mientras que el control biológico natural no prevé ningún tipo de intervención (Begg et al., 2017, Gurr y You, 2016, Heimpel y Mills, 2017).

2.3.2. Control biológico de conservación

La necesidad de transitar hacia un sistema productivo más sustentable en el largo plazo, considerando el agroecosistema en su conjunto, hace que un programa de manejo de plagas que se beneficie de las interacciones y de los recursos ecológicos naturalmente presentes pueda ser clave para obtener una producción agroecológica del cultivo (Deguine et al., 2017). Desde esta perspectiva, el control biológico natural y de conservación adquieren una relevancia primaria. Sin embargo, es necesario considerar que la mayoría de los agroecosistemas están sujetos a aplicaciones de productos de síntesis o vienen de un histórico manejo convencional lo cual provocó disminución de las poblaciones de organismos benéficos, especialmente considerando insectos entomófagos. Como consecuencia de esta situación, el control biológico natural es una opción poco asequible en el corto plazo, pero sí una posible meta futura (Calvo-Agudo et al., 2019, Nicolopoulou-Stamati et al., 2016, Sánchez-Bayo y Wyckhuys, 2019). Por su parte, el control biológico de conservación tomó relevancia en las últimas décadas, con diversos estudios focalizados en aplicar los principios de este tipo de control en los diversos agroecosistemas (Begg et al., 2017).

A pesar del amplio conocimiento relativo al control biológico en todas sus formas, la adopción de estas prácticas de manejo representa un desafío, especialmente al considerar su aplicación en los sistemas productivos. Esto puede ser debido a diversos factores, siendo algunos de ellos: la dificultad en el asentamiento de las poblaciones de los organismos benéficos, la dispersión de la entomofauna benéfica en los lugares aledaños, o el atraso en la recolonización de las áreas de interés, lo cual resulta en una asincronía entre el aumento de las poblaciones de las plagas y la llegada de los enemigos naturales (Alpert, 2006, Begg et al., 2017, Heimpel y Asplen, 2011). Por estos motivos, para poder maximizar los servicios ecosistémicos que pueden brindar los enemigos naturales, y obtener una intensificación sustentable de la producción, es fundamental conocer en profundidad distintos aspectos inherentes a la ecología y a las interacciones que ocurren en los agroecosistemas (Bommarco et al., 2013; Gurr y You, 2016).

Una de las cuestiones fundamentales a tener en cuenta, especialmente cuando se opta por diseñar y desarrollar un programa de control biológico de conservación, es tener conocimiento de los enemigos naturales presentes en el agroecosistema que puedan ser los candidatos para controlar las principales plagas (Begg et al., 2017, Gurr y You, 2016). La incorporación de las técnicas moleculares aplicadas a la ecología de poblaciones permitió en las últimas décadas avanzar de forma significativa en la elucidación de las redes tróficas, con el objetivo de seleccionar los predadores y parasitoides simpátricos que efectivamente se alimentan de las principales plagas, y determinar los momentos de mayor actividad de los entomofagos (González-Chang et al., 2016, Gurr y You, 2016, Prasad y Snyder, 2006).

Si bien elucidar las redes tróficas es un paso fundamental para poder implementar una estrategia de control biológico de plagas mediada por entomofagos, también tener conocimiento sobre otros aspectos de la ecología y etología de los predadores y parasitoides seleccionados es necesario, de manera de poder implementar estrategias que favorezcan la presencia de este gremio en los lugares y en los momentos más propicios (Perović et al., 2018).

2.3.3. Semioquímicos y control biológico

Uno de los aspectos más críticos que conlleva al bajo éxito de los programas de control biológico, especialmente considerando su aplicación en campo, es la dispersión de los controladores naturales y la asincronía entre el aumento de las poblaciones de la plaga y la llegada de los enemigos naturales (Heimpel y Asplen, 2011, Kaplan, 2012). Esto se hace aún más evidente cuando se opta por el control biológico preventivo, que tiene como propósito actuar sobre las primeras generaciones de las plagas, con el fin de evitar los picos poblacionales durante la temporada o durante la época de mayor susceptibilidad al daño del cultivo (Wiedenmann y Smith Jr, 1997). Es en este marco que la posibilidad de manipular aspectos inherentes al comportamiento de estos entomófagos, con el fin de anticipar la llegada de estos a los cultivos, tomó relevancia en las últimas décadas (Kaplan, 2012, Kean et al., 2003).

El comportamiento de forrajeo de un predador o parasitoide pasa por una serie de fases de búsqueda, hasta llegar al encuentro con la presa. Hassell y Southwood (1978) detallan estas fases: la primera es la búsqueda del hábitat, seguida por la elección más a corto alcance del lugar donde están presentes las posibles presas y, finalmente, el encuentro con la presa. Todas estas fases están mediadas por diferentes claves (visuales, acústicas, químicas, etc.). Las claves químicas son las únicas que median las respuestas de largo alcance, como puede ser la elección del hábitat, si bien están involucradas también en el encuentro final con la presa (Fellowes et al., 2005, Heimpel y Asplen, 2011, Mills y Heimpel, 2018).

Estas claves químicas pueden ser producidas por los herbívoros y explotadas por el tercer nivel trófico para rastrear las presas, cumpliendo el rol de kairomonas (Basu et al., 2021, Powell y Pickett, 2003, Qin et al., 2022), o pueden ser producidos por la planta en respuesta al ataque por parte de herbívoros, siendo en este caso sinomonas (Ayelo et al., 2021, Kaplan, 2012, Kelly et al., 2014). Estas últimas, conocidas como HIPVs por su sigla en inglés (herbivore-induced plant volatiles), son metabolitos secundarios usados por el tercer nivel trófico para discriminar los hábitats en los cuales pueden estar presentes las presas de interés, por lo que la posibilidad de usar estos compuestos con el objetivo de anticipar la llegada del tercer nivel trófico, o su permanencia en los lugares de interés, ha sido ampliamente estudiada en diversos sistemas (Aartsma et al., 2017, Dicke y Baldwin, 2010, Dicke et al., 2003, Kelly et al., 2014, Mumm y Dicke, 2010).

2.3.4. Control biológico de la psila del peral

Los insectos pertenecientes al género *Cacopsylla* cuentan con un amplio número de enemigos naturales, tanto parasitoides como predadores, que han sido usados en los diferentes tipos de control biológico. Los predadores, que se caracterizan por ser generalistas, se alimentan principalmente de ninfas de *Cacopsylla.* En el caso de los parasitoides, solo el género *Trechintes* (Hymenoptera: Encyrtidae) se reportó hasta el momento como exitoso en el control de la plaga , cuyo desarrollo también ocurre a expensas de las ninfas de *Cacopsylla* (Booth, 1992, Civolani, 2012, Erler, 2004, Görg et al., 2019, Sanchez et al., 2020, Sanchez et al., 2022, Sanchez y Ortín-Angulo, 2012, Tougeron et al., 2021).

En el ámbito nacional se hizo un relevamiento de la fauna benéfica asociada a la presencia de la plaga. Este estudio demostró que nuestros agroecosistemas cuentan con un gremio biodiverso en el caso específico de los insectos entomófagos. Los predadores registrados pertenecen a las familias *Chrysopidae*, *Anthocoridae, Miridae, Syrphidae y Coccinellidae.* En el caso de los parasitoides, se encontraron diversos morfotipos de *Encyrtidae,* pero las bajas capturas obtenidas durante todos los años de muestreo, hacen suponer que en nuestro agroecosistema no son buenos candidatos para el desarrollo de una estrategia de control biológico (Valle et al., 2021).

Dada la necesidad de generar herramientas de control acordes a un manejo agroecológico de la plaga, se buscó ahondar en una estrategia de control biológico de conservación, buscando favorecer la presencia del gremio de organismos benéficos naturalmente presentes en los agroecosistemas en estudio (Valle et al., 2021). Una de las estrategias de este tipo de control se sustenta en el hecho que tanto predadores como parasitoides necesitan complementar sus dietas con néctar y polen para optimizar su *fitness*. A su vez, para que se puedan establecer en un ecosistema, es fundamental la presencia de presas alternativas que sustenten a los entomófagos en los períodos en los que la abundancia de otras presas son insuficientes (Kean et al., 2003, Wäckers y Fadamiro, 2005).

Fue en esta perspectiva que, en nuestro país, se realizó un trabajo experimental con el objetivo de analizar el efecto del aumento de la biodiversidad vegetal presente en la entrefila y fila de montes de peral en la abundancia de *C. bidens* y el complejo de enemigos naturales reportados, optando por favorecer la flora espontánea presente en nuestros agroecosistemas. Los resultados obtenidos fueron promisorios, se registró una disminución significativa de las poblaciones de la plaga en estudio y un incremento en las poblaciones de artrópodos entomófagos. Esto sugiere que algunos de los predadores encontrados podrían ser buenos candidatos para ser utilizados en un programa de control biológico. También quedó en evidencia que si bien se lograban bajar las poblaciones de forma significativa, la regulación ejercida por parte de los predadores sobre la abundancia de *C. bidens* no permitiría un control satisfactorio (Valle et al., 2021).

Este hecho planteó la necesidad de continuar las investigaciones en el sistema, considerando que para poder ajustar un programa de control biológico es fundamental ahondar en los aspectos ecológicos de los diversos predadores simpátricos.

3. HIPÓTESIS E OBJETIVOS

3.1. HIPÓTESIS

El presente trabajo se desarrolló con el fin de responder a diferentes hipótesis:

1. Algunos de los predadores reportados en los cultivos de peral se sustentan alimentándose de ninfas de *C. bidens.*

2. La llegada de estos predadores es tardía en la temporada, lo que impide un control temprano de las primeras generaciones de la plaga.

3. La planta de peral responde al ataque de *C. bidens* modificando su perfil de volátiles

4. Los predadores utilizan claves químicas para ubicar el hábitat y/o las plantas donde está presente la plaga en estudio.

3.2. OBJETIVO GENERAL

Generar conocimiento sobre las redes tróficas, biología y comunicación química de los enemigos naturales efectivos de *C. bidens*, con el propósito de desarrollar herramientas para la potencial implementación de estrategias alternativas al control químico en el manejo de esta plaga

3.3. OBJETIVOS ESPECÍFICOS

1. Identificar las especies de predadores que se alimentan efectivamente de *C. bidens*

2. Determinar la fluctuación poblacional de los predadores que se alimentan de *C. bidens* y seleccionar al predador más promisorio.

3. Evaluar la dinámica de alimentación de cada uno de estos predadores sobre las diferentes generaciones de *C. bidens* a lo largo de la temporada.

4. Caracterizar los volátiles emitidos por herbivoría de la planta de peral cuando es atacada por *C. bidens.*

5. Determinar si la planta de peral es capaz de modificar su perfil de volátiles de forma diferencial según el herbívoro que se está alimentando. Caracterizar el perfil de volátiles emitido por las plantas de peral según el herbívoro que se alimenta

6.. Caracterizar la respuesta comportamental del depredador *Chrysoperla externa* a los volátiles emitidos por herbivoría

4. WHO IS FEEDING ON THE PEAR PSYLLA? APPLYING MOLECULAR ECOLOGY FOR THE BIOLOGICAL CONTROL OF *Cacopsylla bidens* *

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4.1. RESUMEN

El control biológico es una estrategia alternativa que puede ser usada para el control de *Cacopsylla bidens*. El objetivo de este trabajo fue esclarecer la red trófica que involucra a *C. bidens* como presa. Se emplearon técnicas moleculares y se evaluó la actividad de los diferentes predadores para determinar cuál de ellos se alimenta sobre la plaga en estudio y la dinámica temporal de esta interacción. Cinco especies de predadores resultaron alimentarse sobre *C. bidens*: *Chrysoperla externa*, *Chrysopidae* sp*.*, *Hemerobiidae* sp., *Harmonia axyridis* y *Cycloneda sanguinea*. Todos estos predadores empiezan a alimentarse sobre las primeras generaciones de *C. bidens,* exceptuando *C. externa* que recién empieza a alimentarse sobre la tercera generación de la plaga. Estos resultados permitirán seguir desarrollando estrategias para aumentar la presencia de estos predadores en los montes de peral.

Palabras claves: Control biológico de conservación, Predadores nativos, *Chrysoperla externa*, *Harmonia axyridis, Cycloneda sanguinea.*

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* Artículo publicado en: *International Journal of Pest Management*, 68(4), 390–401.
https://doi.org/10.1080/09670874.2022.2135181

4.2. SUMMARY

Biological control is an alternative strategy to control *Cacopsylla bidens*. The aim of this study was to untangle the trophic network involving *C. bidens* as prey. Molecular techniques along with predator activity surveys were employed to evaluate predation on psylla. Feeding on *C. bidens* was detected for five predators: *Chrysoperla externa*, *Chrysopidae* sp., *Hemerobiidae* sp., *Harmonia axyridis* and *Cycloneda sanguinea*. All these predators begin to feed early in the season, except from *C. externa* which feeds starting from the third psylla generation. These results contribute to the development of strategies to increase the presence of these predators in the orchards.

KEYWORDS: Conservation biological control, Native predators, *Chrysoperla externa*, *Harmonia axyridis, Cycloneda sanguinea.*

4.3. INTRODUCTION

In response to the growing global demand for food, fruit production needs to maximize its productivity. However, yield optimization must be accomplished by the adoption of production methods that ensure long term sustainability and reduce environmental impacts of agricultural management (Cerutti et al., 2011; German et al., 2017; Godfray et al., 2010; Huang, 2004). Fruit production relies on the use of many external inputs, the majority of which are oriented towards pest and disease control, needed to maintain the commercial and aesthetic quality demanded by international standards (Simon et al., 2011). The negative aspects of the use of pesticides are evident both at the human health and environmental levels. In the later, pesticides play a primary role on the reduction of beneficial insects, such as pollinators and biological control agents (Calvo-Agudo et al., 2019; Nicolopoulou-Stamati et al., 2016; Sánchez-Bayo & Wyckhuys, 2019).

In order to reduce the impact of pesticides employed for fruit production, Uruguay has developed a wide-area management program to target the main lepidopteran pests that affect deciduous fruit production, as traditionally these are responsible for most of the pesticide applications (Núñez & Scatoni, 2013). This management program is based on the use of mating disruption and has been successful in achieving a significant reduction in the use of insecticides in fruit orchards (Zoppolo et al., 2016). Such decrease in insecticide applications, has opened the demand and opportunity for controlling the remaining pests by developing other sustainable method such as the use of biological control (Amarasekare & Shearer, 2017).

Cacopsylla bidens (Hemiptera: Psyllidae) is one of the pests that have currently acquired a primary role in pear production, causing both direct damage as well as indirect damage as a vector of the phytoplasma *Candidatus phytoplasma pyri* (Seemüller & Schneider, 2004). Although the control of this pest has been based on the use of insecticides, the possibility of relying on biological control of this pest has gained significance.

In recent decades, due to the successful use of predators and parasitoids in several countries (Erler, 2004; Gajski & Pekár, 2021; Husain et al., 2018; Nin et al., 2012; Sanchez et al., 2022; Shaltiel & Coll, 2004), efforts have been made nationally to explore biological control alternatives for *C. bidens*, resulting in the identification of sympatric beneficial arthropods, mainly generalist predators, with the potential as natural control agents of the genus Cacopsylla (Valle et al., 2021). In this same study, the presence of a possible parasitoid of *C. bidens* was also reported. However, its abundance was very low, suggesting that it was not responsible for reducing the populations of the pest (Valle et al., 2021). Therefore, a biological control strategy to favor the presence of native natural enemies focusing on generalist predators, was considered, as these have proven to be successful, even when pest populations are low (Symondson et al., 2002).

Conservation biological control rely on natural enemies naturally present in the orchards and favoring their existence by providing them with a suitable habitat (including the reduction of the pesticide-induced mortality) for their development. Being different from other models of biological control, as it is not based on the introduction nor mass release of exotic natural enemies (Gurr & You, 2016; Heimpel & Mills, 2017). The ecological intensification of agricultural production can help to enhance the crucial ecosystem function that naturally occurring biological control agents provide, which is the regulation of pest populations and contribution to crop productivity (Bommarco et al., 2013).

To encourage the presence and activity of these natural controllers, it is critical to determine which of these can feed or parasitize the pests that must be controlled (Gurr & You, 2016). In the specific case of *C. bidens* the information is quite scarce, with studies conducted only at the laboratory level or based on the introduction and release of predators and parasitoids (Beninato & Morella, 2000; Erler, 2004; Sanchez et al., 2020; Sanchez & Ortín-Angulo, 2012; Sigsgaard et al., 2006; Tougeron et al., 2021). The development of molecular techniques has allowed for the relatively simple elucidation of trophic linkages within a food chain, allowing the elucidation of the trophic network of many natural enemy guilds in agroecosystems and selection of predator species that could be good candidates for biological control(Agusti et al., 2003a; Alhmedi et al., 2018; Birkhofer et al., 2017; Chen et al., 2000; Clare, 2014; Clare et al., 2009; Colloff et al., 2013; Ekbom et al., 2014; González-Chang et al., 2016; Gurr & You, 2016; Unruh et al., 2016). Although laboratory studies using COI mitochondrial markers to detect predators that can feed on species of the genus *Cacopsylla* have been carried out, there are still no studies at the field level that determine which of these predators present are actually feeding on *C. bidens* (Agusti et al., 2003b).

In order to increase the knowledge of the interactions between predators and *C. bidens,* this study seeks first to determine predator's species associated with the target pest and effectively using it as a food source in the field, and second to determine the influence of each of these predators on the different generations of *C. bidens* throughout the season.

4.4. MATERIAL AND METHODS

4.4.1. Study Site and Field Sampling

All field work was carried out in Melilla (Canelones, Uruguay), the area where most of the country's fruit production is concentrated. Samplings were conducted in five productive pear orchards with crop management practices in line with the nationally-defined integrated management guidelines (DIGEGRA 2018). All the chosen orchards were planted with the same cultivar (Williams). Field samplings were done during three consecutive years, starting in September 2017 until March 2020. During the winter months, from June to August, no samplings were carried out, as only the winter form of adult *C. bidens* in the orchards were found.

With the aim of estimating the presence and the seasonal distribution of the predatory species associated with *C. bidens*, a vacuum suction sampling method was chosen using a manual aspirator (Boda B2001-2.6). The crown of ten trees per orchard were sampled every other week using the aspirator during 10 seconds per sample.

To estimate the number of generations of the pest, a degree-day model was used, assuming the minimum development threshold for *C. bidens* at 6° C. According to reports from previous studies conducted under local conditions, 366 degree-days are needed to complete one generation of *C. bidens* (Mujica, 2014). All climatic data were obtained from the agroclimatic station of INIA Las Brujas (34°40’11.24” S, 056°20’20.43” W).

For the predator gut-content analyses all identified predatory species were hand collected from the crown of 10 trees, from September to May for three years. The collected predators were immediately individualized in plastic tubes in the field and stored in an icebox until arriving at the laboratory where they were stored at -80 °C.

The species-level identification of candidate predators was carried out by using the dichotomous keys according to the different sampled families. (BieNkowski, 2018; Del G. da R. Celli et al., 2021; González et al., 2011; Jouveau et al., 2018; Savoiskaya & Klausnitzer, 1973; Triplehorn et al., 2005). In order to identify cryptic species of Chrysopidae, DNA was extracted, and the 650 bp gene region of COI for DNA was amplified and sequenced as detailed in the following section.

4.4.2. Molecular detection of *Cacopsylla bidens* in predator guts

Total DNA was extracted from the whole body using two different protocols depending on the purpose of the sample: for the molecular gut content analysis the Salting Out protocol was used (Sunnucks and Hales 1996); whereas to test for primer specificity DNA extraction using the Qiagen DNeasy Blood & Tissue Kits was carried out. Prior to DNA extraction, all specimens were individually washed following the Greenstone et al. (2012) protocol to avoid any type of environmental contamination. DNA was amplified by PCR using specific primers for the mitochondrial gene cytochrome C oxidase subunit 1, for the genus Cacopsylla (Cp3F: 5′-GTCTAGTAGACCAAGGAGTAGG-3′ and Cp6R: 5′-CTATTGTGTGGAGGTTTCTTC-3′), which amplified a 188 bp product (Agusti et al. 2003b). Amplification was carried out in a total volume of 20 µl containing 1× DNA polymerase buffer (BiolabsRinc), 2.5 mM MgCl2, 250 µM each dNTP, 10 pmol each primer, 1.25 U of Taq DNA polymerase, and 50 ng of template DNA. Amplifications were performed in an automated Applied Biosystems Veriti™ Thermal Cycler with an initial denaturation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 20 s 50 °C for 30 s, 68 °C for 1 min and final extension at 68 °C for 3 min. For each amplification run, a non-template control and a positive control (target DNA) was added. PCR products (3 µl) were visualized in 1% (w/v) agarose gels in 0.5X TBE buffer stained with a GoodViewTM nucleic acid stain and visualized under UV light.

In order to determine the specificity of the primers and avoid cross amplifications, the DNA of two individuals of each predator was extracted in addition to the DNA of ten aphids, these being the main alternative prey of the collected predators. In this case, the DNA of the predators was extracted only from a single leg, while in the case of the aphids, the DNA of the entire body was extracted. All PCRs were conducted as per the above-mentioned conditions.

4.4.3. Feeding experiments

Feeding experiments were carried out in order to determine the prey detectability half-lives in the gut content of the two most abundant predators, *Harmonia axydiris* (2nd instar) and *Chrysoperla externa* (3rd). *Harmonia axydiris* larvae were collected in the field and left to starve for 72 h, while *C. externa* larvae were provided by a local biocontrol company, Biofabrica®. Each predator was individualized into a Petri dish and left to starve for 72 h. After this time, one 3rd instar *C. bidens* nymph was offered and kept under observation until each predator had consumed the prey. The predators that had not consumed the prey in one hour were discarded from the experiment and used as negative controls. At 0, 3, 6, 9, 12, 18 and 24 h post-feeding four *C. externa* larvae and two *H. axidiris* larvae from each interval were removed from the Petri dish and frozen at −20° C, then maintained at the same temperature until DNA extraction and PCR analysis. Both the extraction and the PCR conditions were the same as previously reported.

4.4.4. Molecular identification of cryptic species of Chrysopidae

To identify cryptic species of Chrysopidae, DNA from a single leg of 20 larvae were extracted using the Qiagen DNeasy Blood & Tissue Kit. Universal DNA primers were used to amplify the gene region COI, LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HC02198: 5′-TAAACTTCAGGGTGACCAAAA AATCA-3′ (Folmer et al. 1994). The obtained DNA were sent to Sinsoma GmbH (Voels, Austria) for DNA barcoding following sequencing and alignment based on publicly available sequences found in the databases from NCBI and BOLD, in order to identify the species.

4.4.5. Statistical analysis

In order to evaluate the population fluctuation of both the predators and the target pest, and the possible variation in abundance comparing the three years of sampling, a Generalized Linear Model with the sampling year as an explanatory factor were used. Due to the nature of the data, a Poisson distribution was assumed, and to compare the different sampling years a Post-hoc Tukey analysis was performed ($p < 0.05$). In order to compare the population dynamics of the predators through the year, a Generalized Linear Model sort by month was used, assuming a Quasi-Poisson Distribution due to the overdispersion of the data, with a Post-hoc LSD-Fisher analysis using the agricolae package (De Mendiburu and Yaseen 2020) with a p value < 0.05.

Data from the molecular gut content was first analyzed using a General Linear Model assuming a Quasi-binomial distribution to determine differences in the proportion of feeding detections using lme4 package (Bates et al. 2015), with a Post-hoc Tukey analysis ($p < 0.05$). Second, the proportion of feeding detections was analyzed by comparing the three sampling years using the same GLM model, but assuming a binomial distribution of the data with the sampling years used as an explanatory factor. The last step was to analyze the difference in the feeding detection proportion obtained by the different families of predators, comparing them to the different generations of the pest under study. GLMs were used again assuming a binomial distribution with Psylla generation and the sampling year as fixed explanatory factors.

All analyzes were carried out using the statistical software R Core Team (2022).

4.5. RESULTS

4.5.1. Field sampling

A total of 3653 predatory insects were collected from the crown of the trees during the 3 years of sampling. The most abundant predatory group was Coleoptera, with a total of 531 individuals, all belonging to the family Coccinellidae and distributed among three species. The most frequently found species was *Harmonia axyridis* (210), followed by *Cycloneda sanguinea* (196) and *Adalia bipuntacta* (125). The second most frequent group was Neuroptera, with a total of 470 individuals collected. Among these, three species were found: *Chrysoperla externa* being the most abundant with 241 individuals collected, followed by a second undetermined species of the family *Chrysopidae* (71) and a third undetermined species of the family Hemerobiidae (158), both identified only to the family level. Finally, only a few individuals belonging to the heteropteran family Miridae (86) and the dipteran Syrphidae (16) were captured with one and two species, respectively.

Comparing the three years of sampling, *Cycloneda sanguínea* was more abundant during the first year and *Adalia bipunctata* during the third sampling year. Among the Neuroptera, only the undetermined species from the family Hemerobiidae showed significant differences between years, with the lowest abundances during the second sampling year. The other predators showed similar abundances during the three years of sampling (Figure 1).

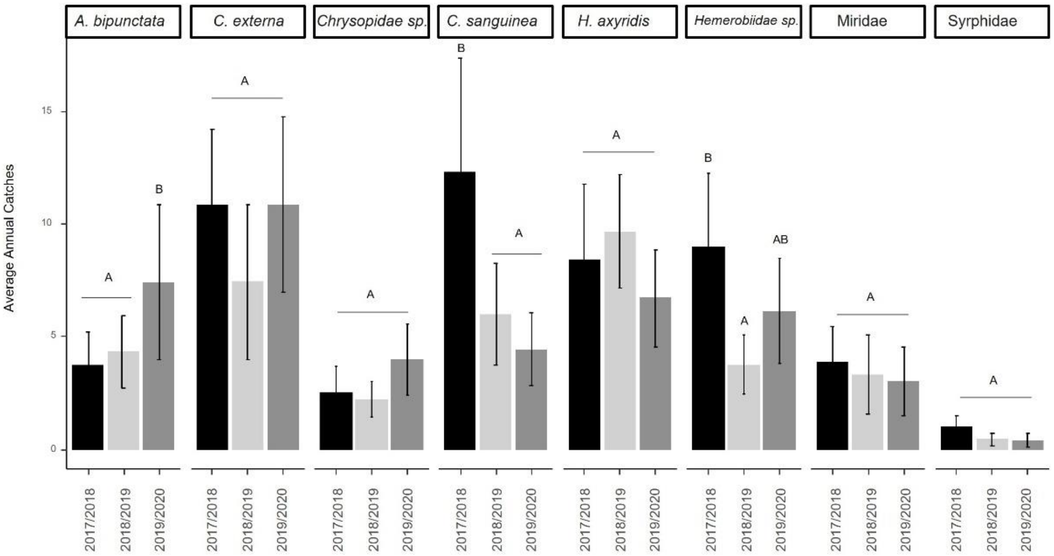

Figure 1. Comparison of the average captures per year of each predator. Different letters represent statistical differences among years for a single predator species ($GLM_{poisson}$ with Tukey $p < 0.05$).

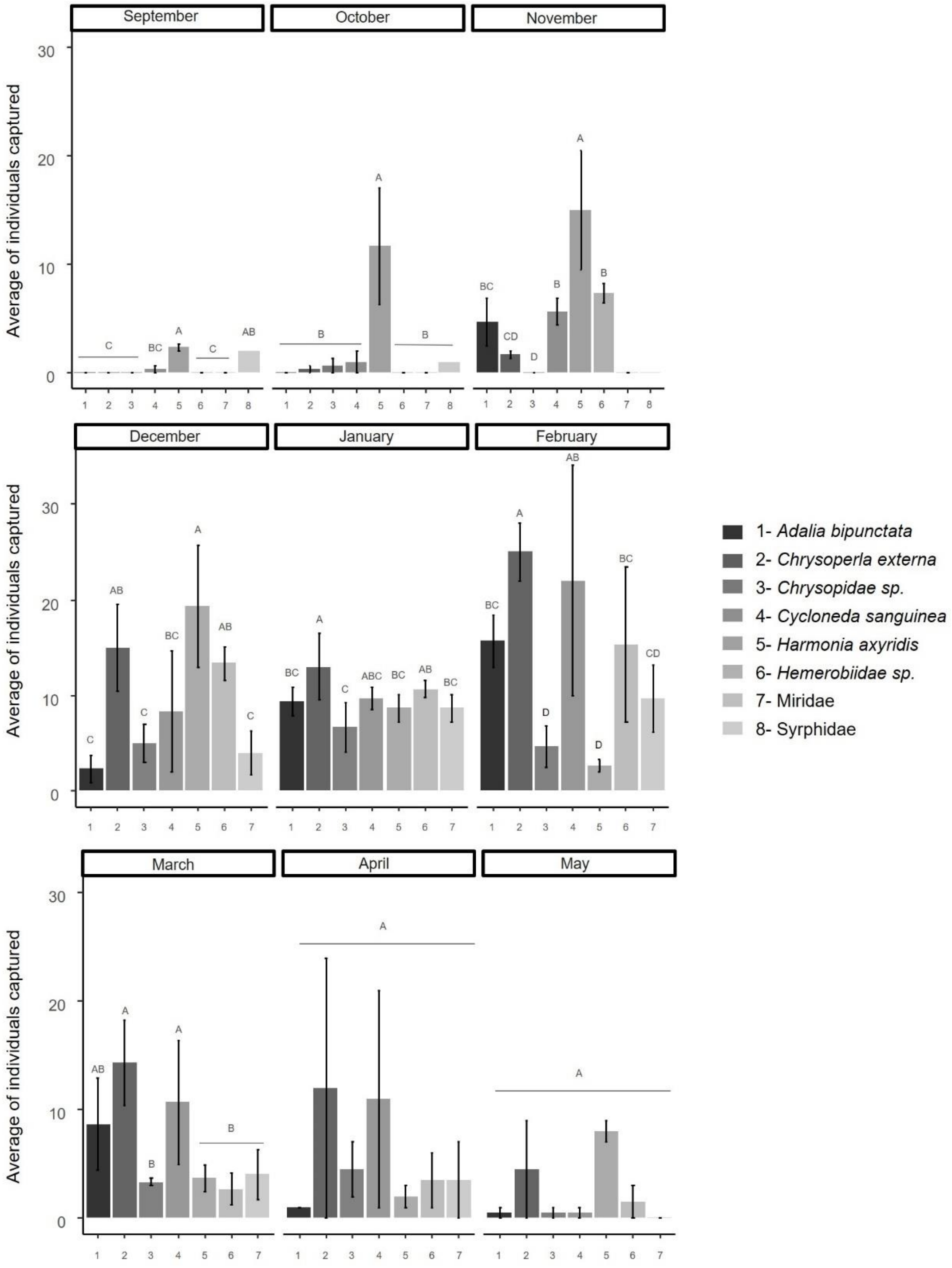

Figure 2. Comparison of the population dynamics in average catches obtained in the three years of sampling, sort by months of the different species of predators found. Different letters represent statistical differences among month for the predator's species ($GLM_{quasipoisson}$ with LSD-Fisher, $p < 0.05$).

Analyzing the population dynamics of these predators throughout the year, we observed that during early spring the presence of predators were very low, with only a few individuals of *C. sanguinea* and *H. axyridis*. Throughout the spring, abundances of *H. axyridis* was on the increase, particularly during October. By late spring (December in the southern hemisphere) all predators were present. Neuroptera species appeared during October, then gradually increased throughout the season until reaching a population peak in February for *Chrysoperla externa*, along with with *C. sanguinea* (Figure 2).

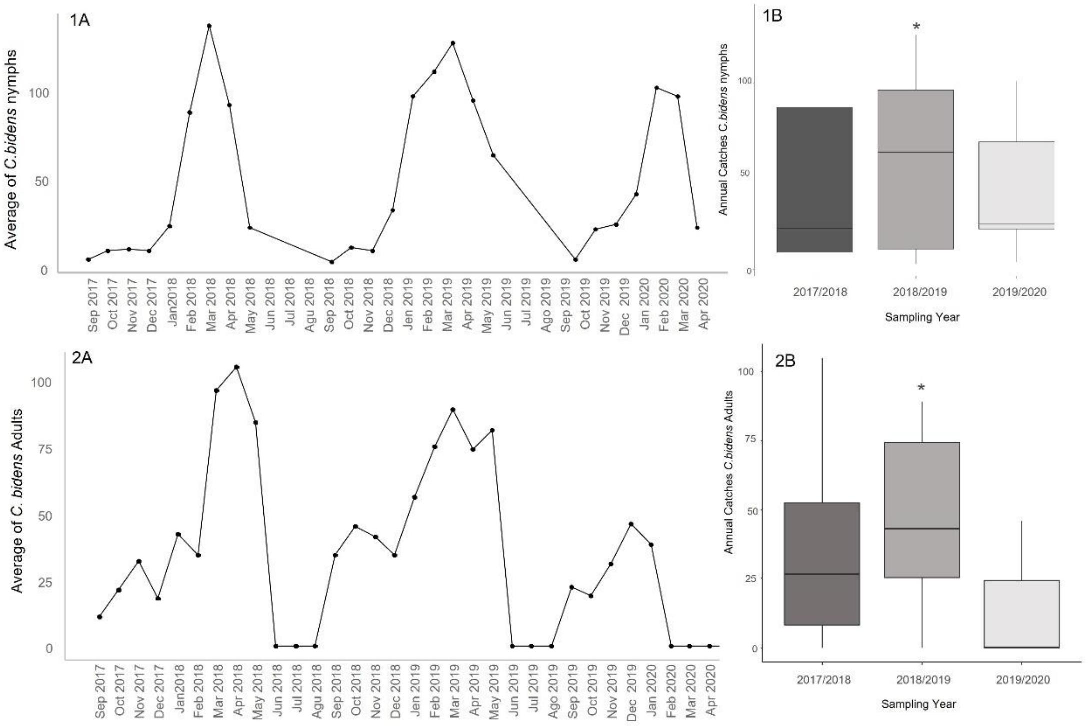

Figure 3. Seasonal distribution of *Cacopsylla bidens* nymphs (1A) and *Cacopsylla bidens* adults (2A). The values shown are the average of the three years of sampling sort by month. Boxplots showing the variation in total catches in the three years of nymphs (1B) and adults (2B). The asterisk marks statistical differences among years ($GLM_{poisson}$ with Tukey, $p < 0.05$).

A total of 1244 *Cacopsylla bidens* nymphs and 1306 adults were captured, showing an uneven distribution among the three years (Figure 3). The second year recorded the highest abundance of the pest (both as nymphs and adults). The seasonal distribution during these three years maintained the same pattern, resulting in a greater abundance of the pest, both of immature and adults, during the months of February, March and April, with a last peak of adults of the winter form in May. According to the information reported by Mujica (2015), *C. bidens* needs 366 degree-days to complete one cycle. Considering a minimum development threshold of 6° C, during the three years of evaluation *C. bidens* completed eight generations per year.

4.5.2. Molecular gut-contents

Table 1 details the specimens collected and tested for each morphotype. Immature stages of Neuroptera, adults and larvae of Coccinellidae, adults of Miridae and larvae of Syrphidae were all selected based on the predation capacities of each family. A total of 701 individuals were tested to determine the presence of *C. bidens* remains in their guts.

The primers used for amplification showed appropriate specificity, since two individuals for each predator and each aphid tested, showed no positive amplification results (false positives). The detection threshold of the primers was 1.2 pg (10^{-4} dilution). The Chrysopidae specimens sent for sequencing turned out to belong to the species *Chrsyoperla externa* and a second morphotype that could not be determined at the species level. Sequenced *C. externa* COI fragments were deposited in GeneBank under the accession numbers from ON360000 to ON360019. Digestibility tests were carried out with *C. externa* larvae, showing feeding detection up to 24 h after feeding, while the feedings experiments with *H. axyidiris* showed that 18 h post-feeding no amplicons were detected.

Table 1. Hand-collected predators in the three years and analyzed by molecular techniques to detect traces of *Cacopsylla bidens* in their gut.

Species	Positive	Negative	Totals	Frequency
Chrysoperla externa	109	34	143	76,2 A
Chrysopidae sp.	21	46	67	31,3 B
Hemerobiidae sp.	42	85	127	33,1 B
Harmonia axyridis	29	101	130	22,3 B
Cycloneda sanguinea	32	66	98	32,7 B
Adalia bipunctata	0	95	95	0,0 C
Miridae	0	19	19	0,0 C
Syrphidae	0	22	22	0,0 C
Totals			701	

Different letters represent statistical differences ($GLM_{quasibinomial}$ with Tukey, $p < 0,05$).

Of the eight species of predators collected, we detected feeding on the target species in five of these. In the case of Coccinellidae, the feeding detection for larvae and adults did not show differences, and therefore were not separated in the reported data (Table 1). *Chrysoperla externa* turned out to be the predator with the highest detection frequency, followed by the other undetermined Chrysopidae species and by *Cycloneda sanguínea*, *Harmonia axyridis* and *Hemerobiidae sp*., all of which showed no differences in detection frequency per sampled species (Table 1). These results did not change between years, with *Chrysoperla externa* showing significant larger detection frequencies than all the other predators. Only during the last sampling year there was a slight increase in the detection frequency of *Chrysopidae sp.* with no significant differences with *Chrysoperla externa* ($p < 0.05$) (Figure 4).

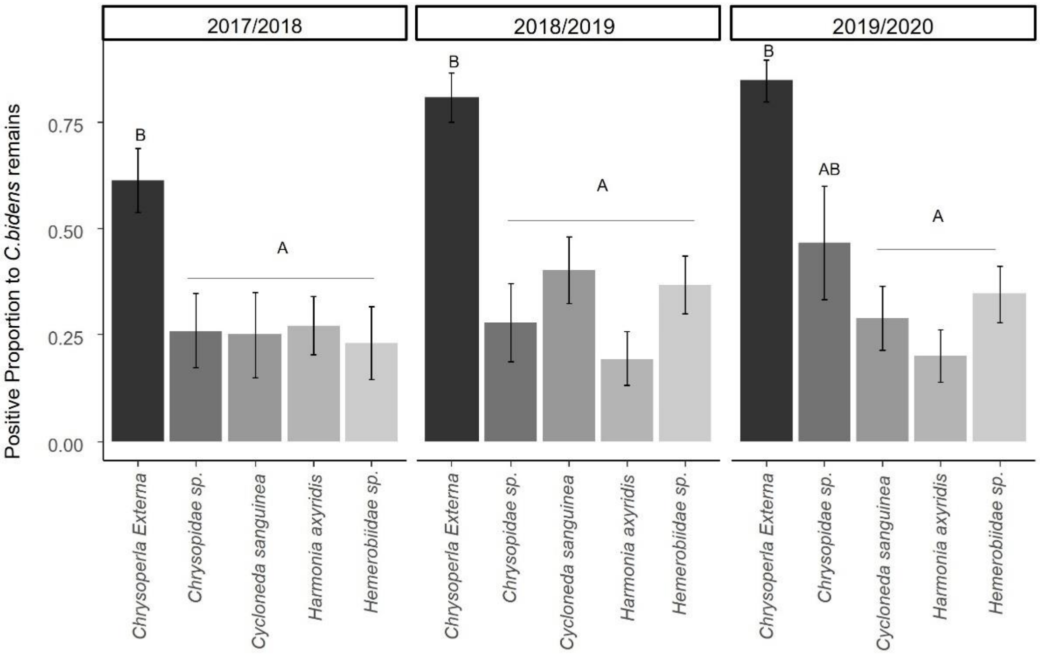

Figure 4. Proportion of individuals that showed traces of *Cacopsylla bidens* remains in their stomachs and considered positive for feeding on *Cacopsylla bidens*. The proportions of positivity in each year for each species of predator are compared. Different letters represent statistical differences ($GLM_{binomial}$ with Tukey, $p < 0.05$).

Predator abundances varied during the seasons in all three sampling years. For all sampling years, *C. bidens* began its activity by the end of August (mid to late winter), completing its first generation by mid-October. Considering the 366 degree-days needed to complete one generation, the subsequent generations were completed in an average of 30 days, with overlapping generations in a single month during the hottest periods of the summer. In this way, the second generation was completed during November, the third during December, the fourth and fifth during January, the sixth during February, the seventh during March and the eighth generation during April. During the first and second generations of *C. bidens,* the only predators detected feeding on the pest were *C. sanguinea*, *H. axyridis* and the *Hemerobiidae sp.* These predators continued to be present and active during the rest of the season, but during the third psylla generation, it was *C. externa* which showed the greatest detection frequency of *C. bidens*. It was during this generation and subsequent generations that the other species of Chrysopidae showed increased detections, however in lesser proportions compared to *C. externa*, except during the last sampling year (Figure 5).

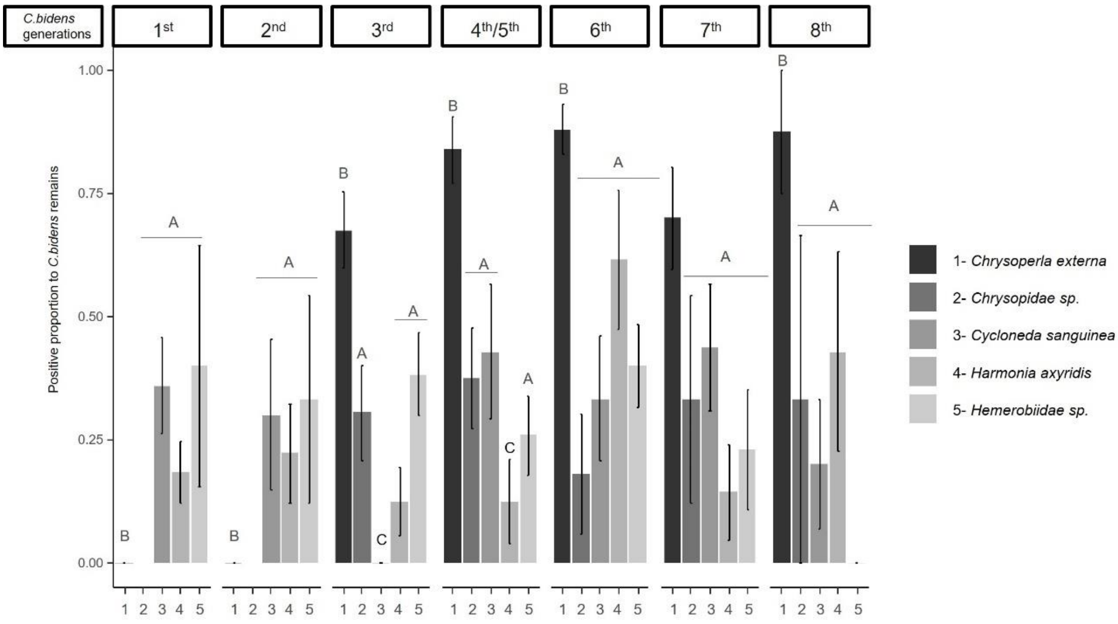

Figure 5. Proportion of individuals that showed traces of *Cacospylla bidens* remains in their stomachs and considered positive for feeding on *Cacopsylla bidens*. The proportion of positivity for each predator was compared sort by each generation of *Cacopsylla bidens*. Different letters represent statistical differences among every generation for the predators species ($GLM_{binomial}$ with Tukey, $p < 0.05$).

4.6 DISCUSSION

This study is the first to use molecular techniques to elucidate the trophic networks for *C. bidens* at the field level, thus determining the pool of predators that are effectively feeding on *C. bidens* throughout the growing season. The results obtained allow us to demonstrate that our pear-orchard agroecosystem contains several generalist predators, which may be good candidates for adopting a conservation biological control strategy. These predators have been present during the three years of sampling, and although a greater presence of *C. bidens* was recorded during the second year, this variation did not influence the populations of the predatory guild. The predators collected were similar to those obtained in previous field surveys, with the only difference that the Miridae species collected were fewer than those previously reported (Valle et al. 2021). This fact can be explained because in the previous studies the interrow of the orchard was sampled, while for this study only the fauna located in the crown of the tree was analyzed. Therefore, many of these previous described mirid species associated with the pear agroecosystem, may be predators of other invertebrates.

The predators in which DNA remains were detected, belong to two orders: Neuroptera and Coleoptera, with the green lacewing *Chrsyoperla externa* showing highest rates of detections for *C. bidens* remains. Furthermore, *C. externa, H. axydiris* and *C. sanguinea* corresponded to the most abundant predators during the three years in study. The proportion of detections of *C. bidens* in these predators was not modified by an increase in the presence of *C. bidens* during the second year, suggesting that there is a weak functional response of this guild to the abundance of the prey. However, as detectability over a minimum threshold and predation strength are not equivalent under molecular gut detection, predators could respond by increasing the consumption of prey per species, without increasing the general frequency of individuals with detections of *C. bidens*.

The activity of Chrysopidae species as biological controllers has been well documented, with several systems showing the presence of lacewings associated with different insect prey pests. Furthermore these predators are among the most commonly mass-reared insects worldwide for their use in augmentation and classic biological control programs (Alcalá Herrera et al. 2022; Tauber et al. 2000). These lacewings are characterized by feeding on a wide range of prey, generally soft-bodied, and in some studies, their association with the presence of psyllids belonging to the genus *Cacopsylla* has already been reported (Albuquerque, Tauber, and Tauber 1994; DuPont and John Strohm 2020; Husain et al. 2018; Rendon et al. 2021; Tianna DuPont et al. 2021; Valle et al. 2021). Moreover, psyllids are known to produce a large amount of honeydew, with which the nymphs cover themselves (Le Goff et al. 2019) and in some cases this honeydew could disrupt predation. On the contrary, psyllid's nymphs are an ideal food source for Chrysopidae, providing the necessary carbohydrates to improve fitness. (Hogervorst et al. 2008; Le Goff et al. 2019). For the agroecosystem under study, it was possible to conclude that the prevalent species is *C. externa* followed by the unidentified *Chrysoperla sp.* Furthermore, *C. externa* had the best detection feeding results, making it the best candidate to improve a biological control strategy. Among the Coccinellidae, several examples used as biological control agents are also reported. Although they are generally associated with aphids, reports of their activity on psyllids have also been made based on direct observation or through correlations. In contrast to what was reported by several authors, that have not reported evidence of feeding by Coccinelidae on the genus *Cacopsylla*, Ge et al. (2019) related H. axyridis as the main predator of *Cacopsylla chinensis*, a species strictly related to *C. bidens*, in East Asian countries (Hodek and Honěk 2009). In the case of *C. bidens*, this is the first report of the feeding activity of two coccinellids, *H. axyridis* and *C. sanguinea*. Although the frequency *C. bidens* predation was low during the three years, the abundances of the predator were similar to *C. externa*, suggesting that these insects would not include the pear psyllid among their preferred prey and that feeding on *C. bidens* may be rather occasional. In the particular case of *H. axyridis*, attention must be paid when managing this species, due to the impact the insect may have on non-target species (Koch 2003). In particular, there are several reports of polyphagia in *H. axyridis*, which has been shown to prey even on chrysopids and other coccinellids (Michaud 2002; Pell et al. 2008; Phoofolo and Obrycki 1998) and therefore could negatively affect the management of this pest.

Because generalist predators are extremely polyphagous, using them in a biological control plan can be challenging and can lead to the failure of the plan (Prasad and Snyder 2006). Several potential predators may be feeding on other predators or parasitoids, and such intraguild predation may negatively affect the efficacy of a biological control strategy (Loomans 2021; Prasad and Snyder 2006; Traugott et al. 2012). In turn, relying on a polyphagous natural controller may bear the advantage that these predators can establish more easily on crops as they are able to survive by feeding on the various prey items that are present (Pekár et al. 2015; Symondson, Sunderland, and Greenstone 2002). In order to resolve this dichotomy, it is essential to thoroughly understand the interactions that occur within the crops, and to analyze the ecological risk associated with each of these natural enemies, this being a key point to avoid the failure of any type of biological control (Loomans 2021).

As the pear-orchard agroecosystem surveyed in our study, hosts not a single predator acting on *C. bidens*, but a complex of natural enemies that may alternate abundances throughout the season, the seasonal dynamics must be considered. Several authors report that the possibility of controlling pests early in the growing season would make it possible to avoid population peaks during the rest of the year, hence decreasing the need to intervene with synthetic products later on (Gajski and Pekár 2021; Pekár et al. 2015). In the specific case of the pear psylla, the first generation begins at the end of August (spring, in the southern hemisphere) with the termination of the winter dormancy of the females and female egg laying (Horton, Higbee, and Krysan 1994; Valle et al. 2021). The fulfillment of the first generation, considering the necessary degree days reported by Mujica (2014), occurred in the month of October. Currently, it is on these early spring generations that it is advised to act with synthetic products, in order to avoid subsequent population peaks and interventions (Civolani 2012). However, these products severely affect their natural enemies population increase, which will affect an early biological control of the pest (Kocourek et al. 2021). In the orchards sampled in our study, the growers routinely practice current integrated production norms, opting for chemical methods at the end of winter. Still, *H. axyridis, C. sanguinea* and *Hemerobiidae sp*. act as early controllers of the first generation of the pest and are likely affected by this intervention, likely explaining the low abundances of these predators in our early-spring samplings. Added to the fact that the activity of *C. externa* begins only when the third psylla generation is underway. It is only around December (summer) that the predator populations begin to increase together with the augmentation of the pest abundance. It is possible that natural biocontrol of psylla populations fail to keep populations under control, due to the low predation pressure early in the season and the asynchrony between the colonization of the crop by the pest and the arrival of beneficial insects. Several authors have highlighted the lack of synchronization of predators with the pest, resulting in a significant increase of the populations during the first generations (Bell 1990; Heimpel and Asplen 2011; Heimpel and Mills 2017; Kaplan 2012; Mills and Heimpel 2018; Settle et al. 1996). This fact takes on even greater relevance as *C. externa* being the main predator feeding on *C. bidens*, is characterized by a type III functional response (Holling 1959; Midon and Idalicia 2021). This type of functional response, is characteristic of predators which decrease the intensity of predation both when the prey are present in very low numbers, or at very high densities, while in intermediate densities it is where they express all their potential as biological controllers (Holling 1959; Real 1977). Under our conditions, the arrival of *C. externa* occurs when the populations of *C.bidens* are increasing significantly, making the neuropteran unable to maintain the populations of the pest under control.

To conclude, in this study we characterized the guild of predators interacting with *C. bidens* as prey. Among these natural enemies, *C. externa* appears as the most promissory predator, although other species in the assemblage may be needed early in the season to control the first generations. More studies are needed on the ecology, behavior and functional responses of these predators, including the range of prey on which they can feed, and determining the risk of intraguild predation. These natural enemies do not seem to be present at abundances high enough to carry out an efficient control of *C. bidens*, therefore management which increases their fitness abundance and synchronicity through a conservation biological control strategy may be the best means to ensures the transition toward an ecological intensification production strategy.

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5. HERBIVORE-DEPENDENT INDUCED VOLATILES IN PEAR PLANTS CAUSE DIFFERENTIAL ATTRACTIVE RESPONSE BY LACEWING LARVAE*

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5.1. RESUMEN

El uso de compuestos orgánicos volátiles (VOCs) para manipular el comportamiento de los enemigos naturales es una herramienta que puede mejorar la eficiencia del control biológico. Entre éstos, los volátiles inducidos por herbivoría (HIPVs) pueden ser usados para atraer o retener los predadores y los parasitoides. Este trabajo tiene como objetivo caracterizar los VOCs emitidos por la planta de peral en respuesta al daño causado por *Cacopsylla bidens*, comparándolos con los VOCs inducidos por un insecto masticador, *Argyrotaenia sphaleropa,* y evaluar la respuesta comportamental de *Chrysoperla externa* a los HIPVs emitidos. Los resultados muestran que las plantas dañadas por la psila del peral emitieron más aldehídos alifáticos respecto a la planta sana. El daño de *A. sphaleropa*, en cambio, resultó en un aumento de liberación de benzenoacetonitrilo, (E)-4,8-dimetilnona-1,3,7-trieno, β-ocimeno y cariofileno. En los bioensayos, las larvas de *C. externa* fueron mayoritariamente atraídas por los HIPVs en comparación con la planta sana. Cuando se compararon los HIPVs emitidos luego del daño por psila respecto al daño por *A. sphaleropa*, *C. externa* fue más atraído por los primeros, mostrando a su vez una mayor probabilidad de respuesta y un tiempo de búsqueda reducido. Estos resultados muestran que la planta de peral responde al daño por herbivoría modificando su perfil de volátiles, y que los HIPVs emitidos por daño de psila puede ser usados como clave por parte de *C. externa*.

Palabras Clave *Cacopsylla bidens · Argyrotaenia sphaleropa · Chrysoperla externa ·* Control biológico · VOCs · HIPVs*

5.2. ABSTRACT

Biological control may benefit from the behavioral manipulation of natural enemies using volatile organic compounds (VOCs). Among these, herbivore-induced plant volatiles (HIPVs) provide potential tools for attracting or retaining predators and parasitoids of insect pests. This work aimed to characterize the VOCs emitted by pear plants in response to attack by *Cacopsylla bidens* (Hemiptera: Psyllidae), a major pest in pear orchards, to compare these with VOCs induced by a leaf chewing insect, *Argyrotaenia sphaleropa* (Lepidoptera: Tortricidae), and to evaluate the behavioral response of *Chrysoperla externa* (Neuroptera: Chrysopidae) to HIPVs from pear plants damaged by either herbivore. The results demonstrated that plants damaged by the pear psylla emitted VOC blends with increased amounts of aliphatic aldehydes. Leafroller damage resulted in increased amounts of benzeneacetonitrile, (E)-4,8-dimethylnona-1,3,7-triene, β-ocimene and caryophyllene. In olfactometer bioassays, larvae of *C. externa* were attracted to herbivore-damaged plants when contrasted with undamaged plants. When plant odors from psylla-damaged were contrasted with those of leafroller-damaged plants*,* *C. externa* preferred the former, also showing shorter response lag-times and higher response rates when psylla-damaged plants were present. Our results suggest that pear plants respond to herbivory by modifying their volatile profile, and that psylla-induced volatiles may be used as prey-specific chemical cues by chrysopid larvae. Our study is the first to report HIPVs in pear plants attacked by *C. bidens*, as well as the attraction of *C. externa* to psyllid-induced volatiles.

Keywords *Cacopsylla bidens* · *Argyrotaenia sphaleropa* · *Chrysoperla externa* · Biological control · VOCs · HIPVs

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[*] Artículo publicado en: *Journal of Chemical Ecology*, https://doi.org/10.1007/s10886-023-01403-8

5.3. INTRODUCTION

Biological pest control, in particular conservation biocontrol, has the potential to support the regulation of pest populations in the agroecosystem and ultimately reduce the use of pesticides (Wyckhuys et al. 2013). However, relying on biological control poses several challenges, especially when the goal is to apply biological control in open field production. Factors that result in low control effectiveness include agricultural practices that disturb the ecological balance of the agroecosystem, natural enemies' dispersal to surrounding areas, delayed return to the crop and the resulting asynchrony between natural enemies and the target pests (Bell 1990; Heimpel and Asplen 2011; Kaplan 2012; Tooker et al. 2020). To overcome these drawbacks, it is critical to have a thorough understanding of the foraging behavior of natural enemies, as well as viable techniques to modify their dispersal behavior and encourage their persistence in the crop.

Foraging behavior of natural enemies, both predators and parasitoids, often encompasses a series of steps that facilitate prey finding. These steps may include chemical, visual, acoustic and/or olfactory cues (Fellowes et al. 2005; Mills and Heimpel 2018), the latter usually associated with prey habitat location (Bell 1990; Fellowes et al. 2005; Lim and Ben-Yakir 2020). Among the chemical cues, natural enemies have the capacity to exploit volatile organic compounds (VOCs) that are emitted by plants after herbivore damage; chemicals usually regarded as herbivore-induced plant volatiles or HIPVs (Ayelo et al. 2021a). These are secondary plant metabolites that may act as induced defenses by repelling or preventing feeding and oviposition (Danner et al. 2011; Holopainen and Gershenzon 2010; Piesik and Wenda-Piesik 2015), as well as indirectly by promoting the efficiency of natural enemies (Dicke and Baldwin 2010; Guo and Wang 2019; Vet and Dicke 1992). Furthermore, plants may discriminate between various herbivores and elicit a differential chemical response according to factors such as the feeding mode employed by insect herbivores (Bidart-Bouzat and Kliebenstein 2011; Danner et al. 2018; Rowen and

Kaplan 2016). Such specificity in the plant's response, which translates into a different blend of volatiles, makes these VOC blends reliable cues that can be used by natural enemies to locate their prey habitat in a complex environment (Aartsma et al. 2017; Dicke and Baldwin 2010; Vet and Dicke 1992).

The possibility of exploiting HIPVs to attract or promote the permanence of the third trophic level in target crops has been studied in diverse systems to improve biological control effectiveness (Ayelo et al. 2021a; Kaplan 2012; Kelly et al. 2014; War et al. 2011). Several studies in fruit orchard systems have been conducted to illustrate the effectiveness of HIPV attraction of natural enemies both in the laboratory and in the field (Drukker et al. 1995, 2000; El-Sayed et al. 2018; Jones et al. 2011, 2016; Pålsson et al. 2022; Scutareanu et al. 1996, 1997). Attraction has been primarily shown by the use of traps baited with synthetic compounds, resulting in increased presence of predators and leading to the conclusion that these compounds may be attractive to the third trophic level (Lucchi et al. 2017; Pålsson et al. 2022).

Pome fruits crops are affected by numerous herbivorous, including key cosmopolitan pests as well as regionally distributed secondary or primary pests. When pheromone-based control techniques such as mating disruption are successfully used for the management of primary lepidopteran pests, secondary pests typically benefit from the reduction in pesticide use (Zoppolo et al. 2015). In the case of pear orchards, the pear psyllid *Cacopsylla bidens* (Hemiptera: Psyllidae) has assumed a role of primary pest (Burckhardt and Hodkinson 1986; Valle et al. 2017). This psyllid causes both direct and indirect damages due to the feeding of nymphs and adults, the incidence of sooty mold and the vectoring of the phytoplasma *Candidatus phytoplasma pyri* (Horton 1999; Seemüller and Schneider 2004). Control strategies for *C. bidens* heavily rely on the use of insecticides, requiring several applications throughout the season, with decreasing effectiveness due to the development of resistance (Berrada et al. 1994; Buès et al. 2003; Civolani 2010, 2012). These problems have prompted the search for alternative strategies to control this pest, with biological control standing as an affordable and sustainable alternative. Indeed, the efficiency of predators to reduce pear-psyllid populations has been widely demonstrated (Daugherty et al. 2007; Sigsgaard et al. 2006; Tougeron et al. 2021, Sanchez et al. 2022).

Efforts have also been focused at the national level to develop a biological control plan for *C. bidens*. In our previous work in this system we sampled and identified the most abundant natural enemies present in our pear orchard agroecosystem, in association with the presence of the pest (Valle et al. 2021). Subsequently, using molecular ecology tools, we assessed the predators that most frequently predate on *C. bidens* and established that the best candidate predator for deployment in a biological control plan was *Chrysoperla externa* (Valle et al. 2022). In the same study, we observed that the populations of *C. externa* larvae increased only during late spring/early summer, thus allowing the first psylla generations to grow undisturbed since early spring (Valle et al. 2022). Late arrival into the pear orchards may be a key factor that prevents *C. externa* from reaching its potential in reducing *C. bidens* populations. *Chrysoperla externa* larvae present a type III functional response, characterized by a sigmoidal response curve at certain prey densities, but a decreasing efficiency of consumption when prey populations increase above a certain level, eventually reaching an asymptote (Holling 1959; Midon and Idalicia 2021).

Finding strategies to attract *C. externa* in pear orchards prompted us to study the HIPVs of pear plants and their role in predator attraction. Information on the induced volatile response of pear plants is scant, which lead us to include a different herbivore guild to evaluate the specificity of the plant’s response. We thus analyzed HIPVs of pear plants attacked by the piercing specialist *C. bidens* and the chewing generalist leafroller *Argyrotaenia sphaleropa* (Lepidoptera: Tortricidae), a common secondary pest of pome fruit in southern South America (Núñez and Scatoni 2013). Moreover, we comparatively evaluated the behavioral response of green lacewing larvae to volatiles of pear plants attacked by both herbivores.

5.4. METHODS AND MATERIALS

5.4.1. Insects

Cacopsylla bidens adults were collected in pear orchards in early spring (September and October) to begin the first laboratory generation, then reared under controlled conditions in a climate room (24 °C, 14 L:10D). Adults were placed in cages containing 1-year-old Williams' pear seedlings grown in 20-litre pots. The plants were replaced weekly with new plants, and the removed ones were placed in a new cage and monitored for adult emergence. Newly emerged adults were either used for the experiments or returned to the breeding cages to maintain the rearing. *Argyrotaenia sphaleropa* larvae were obtained from a laboratory rearing feed with artificial diet as reported in Rehermann et al. (2016), modified by the addition of 300 g of ground apple instead of maize bran. The colony was kept under laboratory conditions at 24 °C and 14 L:10D. *Chrysoperla externa* larvae were provided by a company dedicated to the mass rearing of biological control agents (Biofabrica®, Uruguay). The adults were fed with a solution of water and honey and the larvae were fed with an artificial diet based on chicken liver until reaching the second stage. Prior to the experiments, the larvae were kept under the same ambient conditions as described and fed with *Sitotroga cerealella* (Lepidoptera: Gelechiidae) eggs. Second-stage larvae were used for the bioassays.

5.4.2. Plants

Two-year-old Williams' pear plants grafted on OH-F40 rootstock were kept in 20-litre pots in a greenhouse with anti-insect mesh. The plants used in the experiments had similar vigor and size, with an average height of 160 cm and between 3 and 4 branches. To further minimize the possibility of herbivory prior to our experiments, two applications of fenpyroximate (Danisis®, DM Agro) were carried out for mite control. These applications were done at least one month before using the plants for experiments.

5.4.3. Collection of plant volátiles

Volatiles were collected in a separate greenhouse that contained only the plants under study to minimize cross-contamination. Groups of three plants were transferred to the greenhouse for simultaneous volatile entrainment from a plant branch enclosed in a polyester oven bag. An empty bag was used for a blank collection of background volatiles. Air was pulled using an air sampling pump (1.5 L/min, APEX, Casella Cel, UK), connected to a glass tube filled with adsorbent filter containing 50 mg of Haysep-Q 80/100 (Restek Corp., USA). Volatiles were initially collected for 24 h to obtain VOCs from undamaged plants. After this, the filters and bags were removed, and the same branch was infested with 20 adults of *C. bidens* or 20 L3 larvae of *A. sphaleropa*. The insects and branch were enclosed in a net bag and left for 24 h for the insects to feed, after which a new 24-h VOC collection was done to obtain samples from damaged plants, using the same conditions as above. A total of 14 plants were sampled, 8 plants with *C. bidens* damage in late summer (February-March), and 6 plants with *A. sphaleropa* damage in the early fall (April – May). Because the VOC collection experiments with the two herbivores were conducted in different periods of the season, our analysis only considers the change in VOC composition prior and after herbivore damage for each separate herbivore.

5.4.4. Chemical análisis (GC-MS)

VOCs were eluted from the adsorbent filter with 1 mL hexane and concentrated to 100 µL under a stream of Nitrogen. GC-MS analyses were done in a QP-2010 instrument (Shimadzu Corp.) equipped with a non-polar column (Rtx-5MS) (Restek, USA) (30 m x 0.25 mm, 0.25 µm), operated with a constant carrier gas flow of 1 mL/min. The oven temperature program started at 40 °C for 1 min, then ramped to

280 °C at 10 °C/min and remained at the final temperature for 1 min. The injector and interphase temperatures were 250 and 260 °C, respectively. Injections were done in the splitless mode (1 min sampling), and mass spectra were collected in the scan mode at 70 eV (mass range: m/z 30–350). Identification of VOCs was based on ion fragmentation patterns using the NIST17 Mass Spectral Library and by retention indices. Amounts in individual compounds are shown as relative amounts within the VOC blend, calculated from peak areas.

5.4.5. Olfactometer bioassay

A Y-shaped olfactometer consisting of a glass tube (3.5 cm diam x 34 cm long, with 55° inside angle) was used to evaluate the response of L3 larvae of *C. externa*. The Y-tube was placed horizontally in a white box to block potential visual cues. An artificial light source was used on top of the device, ensuring that the same intensity of light was distributed throughout the device. All the assays were carried out in the same greenhouse where the VOC collections were made. The contrasted stimuli were: (*i*) clean air (empty polyester oven bag) vs. undamaged plant (polyester oven bag covering a healthy pear branch); undamaged plant vs. plant damaged by *C. bidens*; (*iii*) undamaged plants vs. plant damaged by *A. sphaleropa*; (*iv*) plant damaged by *A. sphaleropa* vs. plant damaged by *C. bidens*.

To obtain the stimuli corresponding to damaged plants, one branch of an undamaged plants was infested with 20 adults of *C. bidens* or 20 L3 larvae of *A. sphaleropa*, as described above. After 24 h in which the herbivores were allowed to feed, the damaged plants were connected to the olfactometer. A push-pull air system was used; charcoal-cleaned air was pumped (2 L/min in each stimulus) through polyester oven bags containing one plant branch. At the outlet of the olfactometer, a suction pump (1.5 L/min, APEX, Casella Cel, UK) was placed to ensure the continuous air flow through the olfactometer. All bioassays were performed between 09:00 and 16:00 h at 25 °C, during summer and early fall (February-April). To avoid position bias, the olfactometer arms were rotated after each trial and every 10 replicates both plants, damaged and undamaged, were replaced. Due to the possibility that the HIPVs induced a response in the undamaged plants, these were kept separated until they were used in the olfactometer bioassays.

Lacewing larvae were previously starved for 24 h, then placed at the entrance of the Y-tube. During the first 5 min, the larvae were kept in the acclimatization zone, where they were not allowed to access into the device, but the arrival of air was allowed, via a gauze partition. After this initial period, the gauze was removed, and and they allowed to wander through the device for 15 min, recording the time spent in each section (central arm and the two arms bearing the odorous stimuli), the time required to enter the first stimulus arm, and the first arm chosen. The choice of a stimulus arm was recorded when the larva passed the midpoint of the arm. Larvae that failed to leave the central tube of the olfactometer during the 15-min of the bioassay duration were considered non-responders. For each stimuli comparison, replicates were made until reaching 40 responses (defined as one arm chosen) within the 15-min assays.

5.4.6. Statistical analyses

A *Bray-Curtis* dissimilarity matrix was calculated to determine the discrimination between the blend composition of pear plants before and after herbivore damage by permutational analysis of variance with adonis from “vegan” package, with N = 10,000 permutations. The multivariate homogeneity of dispersion was tested (*betadispr*, Vegan package) (Oksanen et al. 2022). To proceed with the visualization of the *Bray-Curtis* dissimilarity matrix, non-metric multidimensional scaling (*NMDS*) was performed (ggplot2) (Wickham 2016). The differences in relative amounts of individual compounds and compound groups were assessed by *paired t-tests* with 0.05 significance.

For the analysis of behavioral responses, the first arm choice by *C. externa* larvae was analyzed by an *exact binomial test* considering equal probability for each olfactometer arm. To compare the proportion of individuals that did not respond, a general linear model (GLM) was used with a binomial distribution, followed by a *Tukey* post-hoc analysis to compare among treatments ($P < 0.05$). The comparison of the time taken for the first arm choice, defined as the proportion of bioassays time spent before passing the midpoint of the chosen Y-arm, was done with a *GLM* with quasibinomial distribution with a *Tukey* post-hoc analysis (lme4 packages) (Bates et al. 2015). All statistical analyses were preformed using R (version 4.2.1) (R Core Team 2022).

5.5. RESULTS

5.5.1. Herbivore-Induced volatiles in pear plants

Volatile extracts from pear plants damaged by *C. bidens* exhibited 25 compounds identified based on their mass spectra and retention indices (Table 1, Table S1[*]). These compounds were also present in VOC extracts from undamaged plants, although nine of them in significantly different relative amounts (Table 1; Fig. 1). Analyzed as whole blends, VOC profiles from psylla-damaged plants were not significantly different from those of undamaged plants (*perMANOVA*, $F = 2.13$, $df = 1$, $P = 0.06$). The permutational dispersion was homogeneous (*PERMDISP*, $F = 0.53$, $df = 1$, $P = 0.47$) and the *NMDS* ordination allowed to separate the two VOC blends (Fig. 2). Among the 25 volatile compounds, 15 were responsible for the separation between blends from damaged and undamaged plants (Table 1; Fig. 2).

The VOC blend emitted by psylla-damaged plants showed significant increases in the proportion of aliphatic aldehydes ($P = 0.002$) and a single alcohol [(*E*)-2-nonenol, $P = 0.001$] (Table 1). Aldehydes increased more than 3-fold their presence in the blend,

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[*] El material suplementario está publicado en la versión ONLINE del articulo

with nonanal (*P* = 0.03) and decanal (*p* < 0.01) as the major components within this group (Table 1).

Table 1. Relative amounts of VOCs emitted by pear plants prior and after herbivory by *Cacopsylla bidens and Argyrotaenia spahleropa*

Compound class	***Cacopsylla bidens***			***Argyrotaenia sphaleropa***		
compound name	**Undamaged[1]**	**Damaged[1]**	**Permanova[2]**	**Undamaged**	**Damaged**	**Permanova**
Monoterpenes	5.7(± 2.3)^a	5.6(± 2.5)^a		1.2(± 0.5)^a	2.6(± 3.1)^a	
(*Z*)-β-ocimene	4.1(± 1.8)^a	1.2(± 1.4)^a	***	1.8(± 0.7)^a	9.5(± 2.6)^b	**
linalool oxide	-	-		0.5(± 0.2)^a	0.4(± 0.1)^a	
β-myrcene	4.9(± 0.8)^a	5.2(± 0.9)^a		-	-	
limonene	6.9(± 1.6)^a	8.7(± 1.7)^a	*	-	-	
(*E*)-β-ocimene	6.3(± 1.9)^a	6.7(± 2.5)^a		-	-	
α-terpineol	6.7(± 2.7)^a	6.1(± 2.1)^a		2.5(± 1.0)^a	0.0^a	**
Sesquiterpenes	1.8(± 1.2)^a	1.2(± 0.7)^a		5.1(± 2.4)^a	3.3(± 1.6)^a	
α-copaene	5.7(± 1.3)^a	2.6(± 0.8)^b		0.2(± 3.2)^a	0.2(± 0.9)^b	
β-caryophyllene	0.2(± 0.1)^a	0.8(± 0.2)^b		0.0^a	0.04(± 0.2)^b	
α-farnesene	1.6(± 0.6)^a	1.1(± 0.4)^a	**	5.1(± 2.0)^a	5.1(± 1.5)^a	
α-muurolene	1.2(± 0.3)^a	0.6(± 0.1)^b		-	-	
δ-cadinene	2.0(± 0.5)^a	1.5(± 0.3)^a		2.3(± 0.9)^a	1.6(± 0.5)^a	
sesquilavandulyl acetate	0.2(± 0.1)^a	0.7(± 0.2)^a	*	-	-	
Aliphatic aldehydes	1.3(± 0.2)^a	4.3(± 1.6)^b		6.2(± 4.5)^a	7.5(± 2.3)^a	
octanal	0.4(± 0.1)^a	1.7(± 0.4)^b	***	0.9(± 0.4)^a	2.6(± 0.8)^a	
nonanal	5.2(± 1.4)^a	13.3(± 2.5)^b	***	12.8(± 5.0)^a	14.5(± 3.4)^a	*
decanal	1.9(± 0.6)^a	8.9(± 1.8)^b	***	5.2(± 2.1)^a	5.3(± 1.3)^a	
undecanal	0.1(± 0.1)^a	0.9(± 0.2)^b	***	-	-	
dodecanal	0.1 (± 0.1)^a	0.8(± 0.2)^b	***	-	-	
tetradecanal	0^a	0.3(± 0.1)^a	***	-	-	
Aromatics	6.8(± 2.4)^a	5,1(± 1.5)^a		0.5(± 0.4)^a	11.9(± 4.2)^b	

Compound class	***Cacopsylla bidens***			***Argyrotaenia sphaleropa***		
compound name	**Undamaged[1]**	**Damaged[1]**	**Permanova[2]**	**Undamaged**	**Damaged**	**Permanova**
phenyl acetaldehyde	-	-		0.8(± 0.3)^{a}	2.3(± 1.2)^{a}	
benzeneacetronitrile	-	-		0.1(± 0.04)^{a}	20.8(± 6.6)^{b}	***
1,3,5-trimethylbenzene	7.4(± 2.6)^{a}	6.8(± 1.5)^{a}		-	-	
methyl salicylate	6.3(± 2.3)^{a}	3.3(± 2.2)^{a}	**	-	-	
Terpene Ketones	2.6(± 0.7)^{a}	1.5(± 1.3)^{a}		2.5(± 1.9)^{a}	1.9(± 0.7)^{a}	
6-methyl-5-heptene-2-one	0.3(± 0.3)^{a}	0.1(± 0.1)^{a}		0.0^{a}	2.5(± 1.0)^{a}	
6,10,14-trimethyl-pentadecanone	5.0(± 1.5)^{a}	3.0(± 1.0)^{a}		5.0(± 2.0)^{a}	1.4(± 0.4)^{b}	*
Green leaf volatiles	16.15(± 4.7)^{a}	10.09(± 5.8)^{a}		44.6(± 18.2)^{a}	14.2(± 4.3)^{b}	
(*Z*)-3-hexenyl acetate	28.8(± 5.8)^{a}	18.1(± 6.4)^{a}	**	44.6(± 18.2)^{a}	14.2(± 4.3)^{b}	**
(*Z*)-3-hexenyl benzoate	3.5(± 1.7)^{a}	2.1(± 1.8)^{a}	*	-	-	
Aliphatic alcohols	0.3(± 0.1)^{a}	1.1(± 0.2)^{b}				
(*E*)-2-nonenol	0.3(± 0.1)^{a}	1.1(± 0.2)^{b}	**	-	-	
Homoterpenes	5.3(± 1.4)^{a}	4.0(± 1.3)^{a}		0.7(± 0.3)^{a}	10.4(± 2.9)^{b}	
(*E*)-4,8-dimethylnona-1,3,7-triene	5.3(± 1.4)^{a}	4.0(± 1.3)^{a}	*	0.7(± 0.3)^{a}	10.4(± 2.9)^{b}	**
Not Identified				4.6(± 1.8)	3.5(± 1.6)	
NI (7.9 min)	-	-		6.0(± 2.5)^{a}	4.4(± 2.0)^{a}	**
NI (18.8 min)	-	-		3.6(± 1.5)^{a}	0.2(± 0.1)^{a}	**

[1] Data shown as percent area within each VOC sample [mean (± SE)]. Different letters indicate significant differences between undamaged and damaged plants within a single herbivore and compound (*paired t-test*, P<0.05).

[2] Asterisks denote compounds with significant influences in the permutational analysis of variance (PERMANOVA) used to analyze the separation between damaged and undamaged plants within each herbivore (*: P<0.05; **: P< 0.01; ***: P<0.001).

Sesquiterpenes showed some significant variation (increase and decrease) as individual compounds, but not as group. Monoterpenes, green-leaf volatiles and aromatics did not show significant variations due of psylla damage, although they comprise a combined 75% of the VOC blend (Table 1).

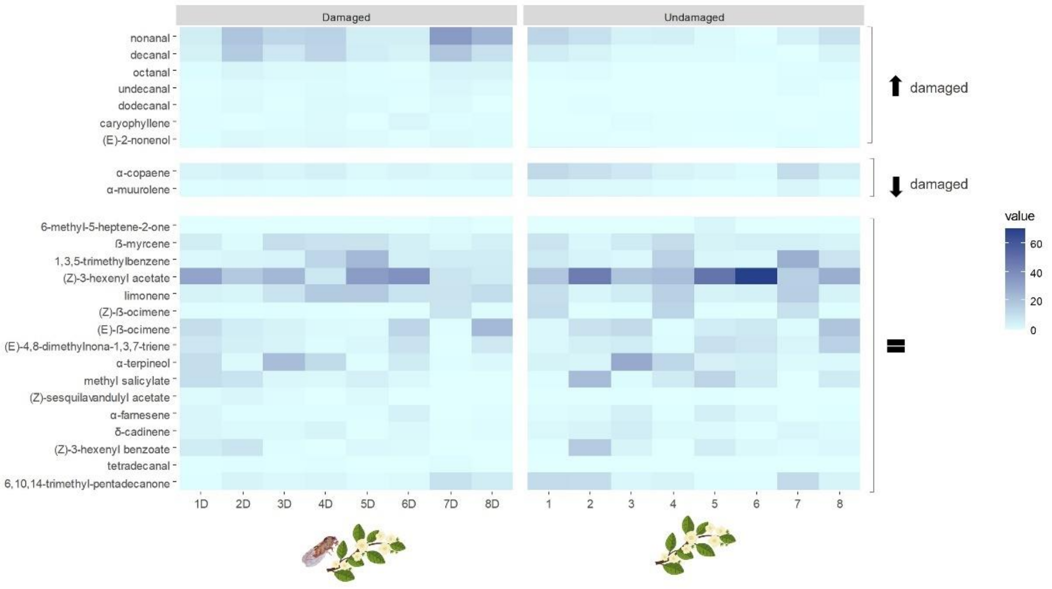

Fig. 1 Heatmap visualization of the relative blend proportions of VOCs from pear plants after *Cacopsylla bidens* damage, in comparison with VOCs from the same plants prior to psylla damage (N = 8 plants). Color scale shows area percent. The up-arrow groups the compounds that increase after damage, the down arrow those that decrease, and the equals sign those that unvaried (*paired t-Test*, *P* < 0.05)

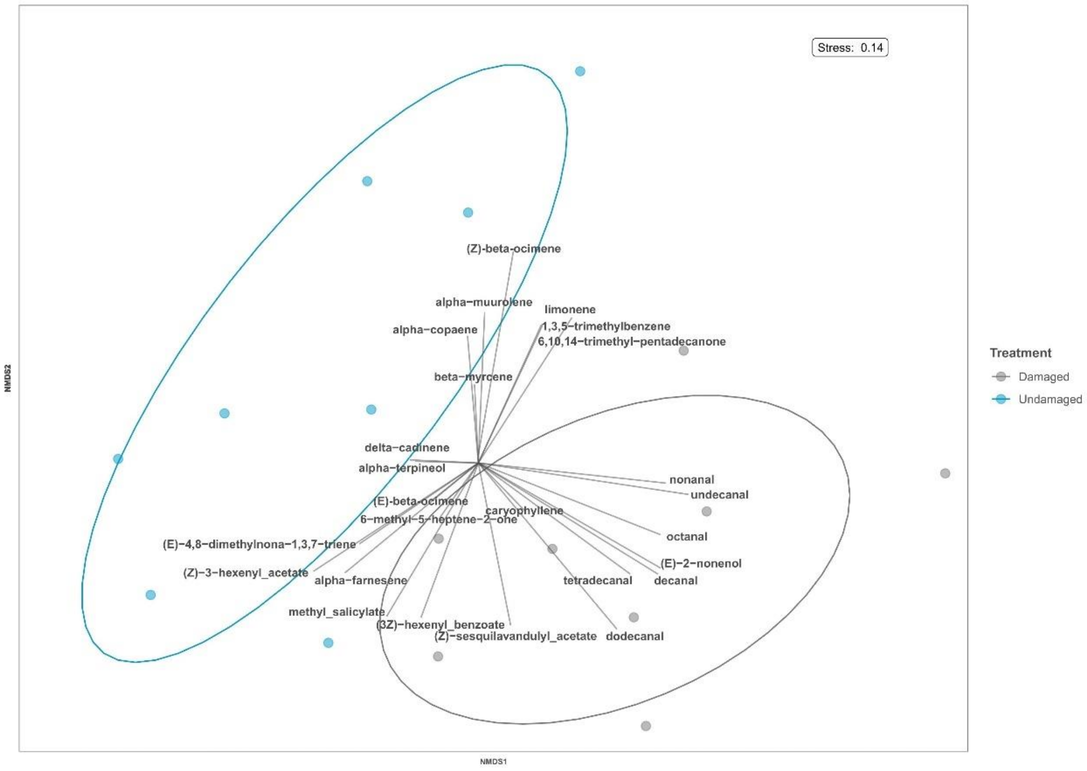

Fig. 2 Visualization of non-metric multidimensional scaling (NMDS) ordination based on *Bray-Curtis dissimilarities* of VOCs emitted from undamaged and psylla-damaged pear plants (N = 8 plants)

Pear plants damaged by *A. sphaleropa* showed a different VOC induction pattern. Volatile analysis showed 21 compounds (Table 1, Table S1), present both in undamaged and damaged plants. Four of these significantly increased their contribution to the blend as a result of leafroller damage, while three decreased their proportions (Table 1; Fig. 3). As a whole, the volatile profiles of undamaged and leafroller-damaged plants differed significantly (perMANOVA, *F* = 7.20, *df* = 1, *P* = 0.008). This difference is also reflected in the NMDS representation, with a clear separation between the profiles of undamaged and damaged plants (Fig. 4).

The permutational dispersion was homogeneous between the groups (PERMDISP, *F* = 0.16, *df* = 1, *P* = 0.69). Of the 21 compounds, 10 contributed significantly to the separation between the two blends (Table 1; Fig. 4). Considering compound classes, leafroller damage induced higher emission of aromatic compounds (*P* = 0.02) and the homoterpene (*E*)- 4,8-dimethylnona-1,3,7-triene (DMNT), which increased 15-fold (*P* = 0.02) (Table 1). At the individual compound level, the increase in aromatics was exclusively due to a remarkable 200-fold increase in the relative proportion of benzeneacetonitrile (*P* < 0.05). Terpenes did not show significant variation as compound classes, although (*Z*)- β-ocimene showed a significant 5-fold increase (Table 1). Finally, the green leaf volatile (*Z*)-3-hexenyl acetate and the terpene ketone 6,10,14-trimethyl-pentadecanone showed significant reductions in their blend proportion in damaged plants (Table 1).

Blend composition changes induced by herbivory by *C. bidens* and *A. sphaleropa* was accompanied by an overall increase in volatile total amounts. Using an internal standard, we calculated VOC total amounts emitted in 24-hour volatile entrainments. These net amounts were multiplied by 3.9 ± 3.3 and 2.8 ± 1.3 (mean ± SE) after damage by *C. bidens* (N = 8) and *A. sphaleropa* (N = 6), respectively, in comparison with the same plants before insect damage

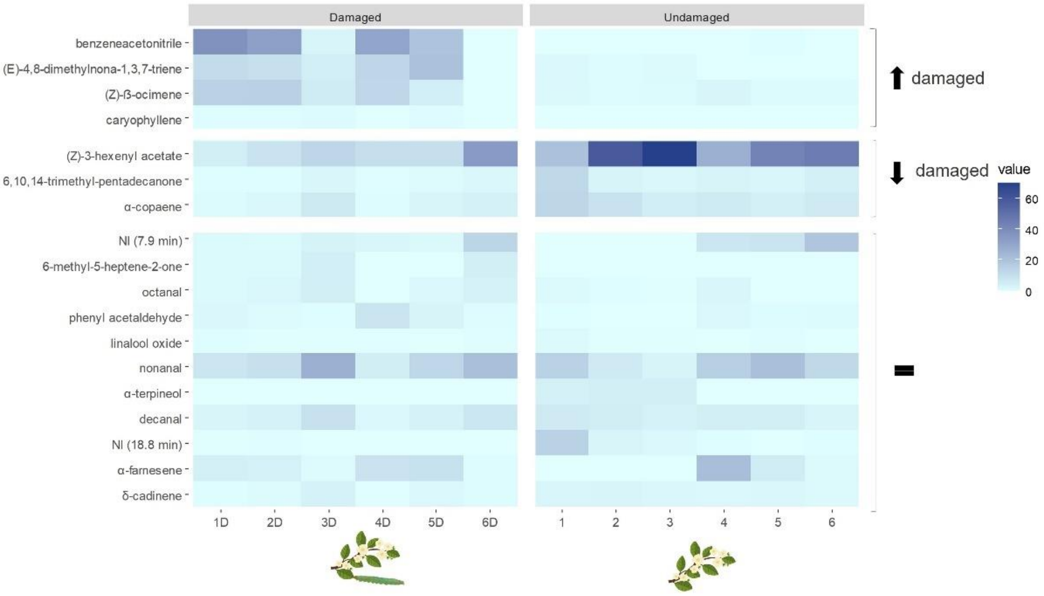

Fig. 3 Heatmap visualization of the relative blend proportions of volatile compounds from pear plants after *Argyrotaenia sphaleropa* damage, in comparison with VOCs from the same plants prior to leafroller damage (N = 6 plants). Color scale shows area percent. The up arrow groups the compounds that increase after damage, the down arrow those that decrease, and the equals sign those that unvaried (*paired t-Test*, *P* < 0.05)

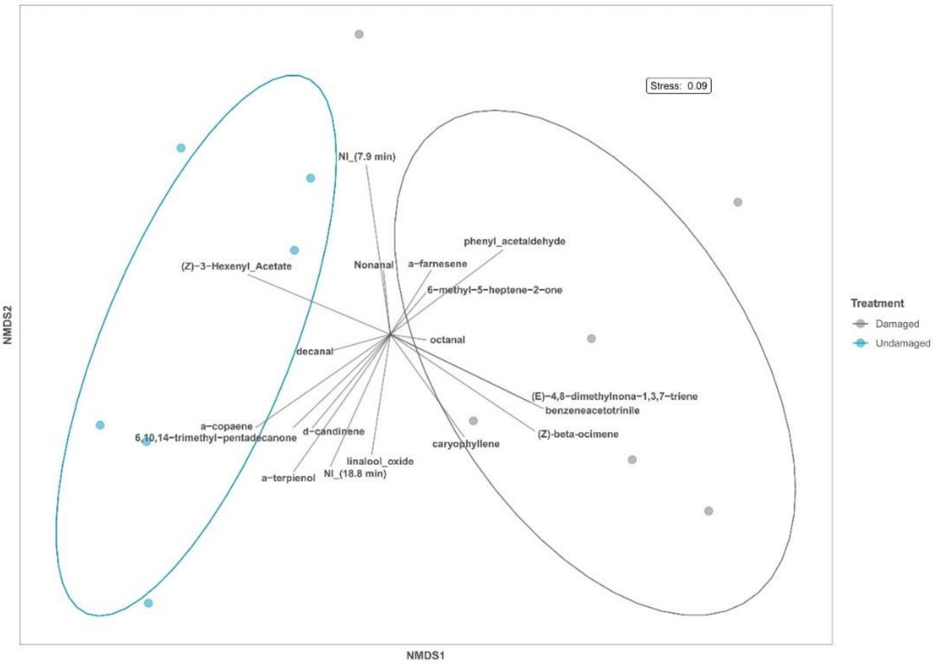

Fig. 4 Visualization of non-metric multidimensional scaling (NMDS) ordination based on *Bray-Curtis dissimilarities* of the volatiles organic compounds emitted from undamaged and leafroller-damaged pear plants (N = 6 plants)

<u>5.5.2. Behavioral response of chrysopid larvae</u>

The search behavior of *C. externa* larvae was quite consistent throughout the olfactometer bioassays. Once the larva chose one arm, it remained in the chosen arm for the rest of the assay time, so first arm choice was clearly the most significant variable to measure in the bioassays. Nonetheless, two other parameters related to food-search motivation were analyzed; the time spent in the non-choice area before selecting one arm, and the proportion of non-responsive insects. The results obtained showed that *C. externa* larvae were attracted to plants damaged by *C. bidens* and *A. sphaleropa* ($p < 0.05$) when odors from undamaged plants were offered as alternatives in dual-choice bioassays (Fig. 5). When odors from plants damaged by either herbivore were contrasted, psylla-damaged plants were more attractive than leafroller-damaged plants ($p = 0.01$) (Fig. 5).

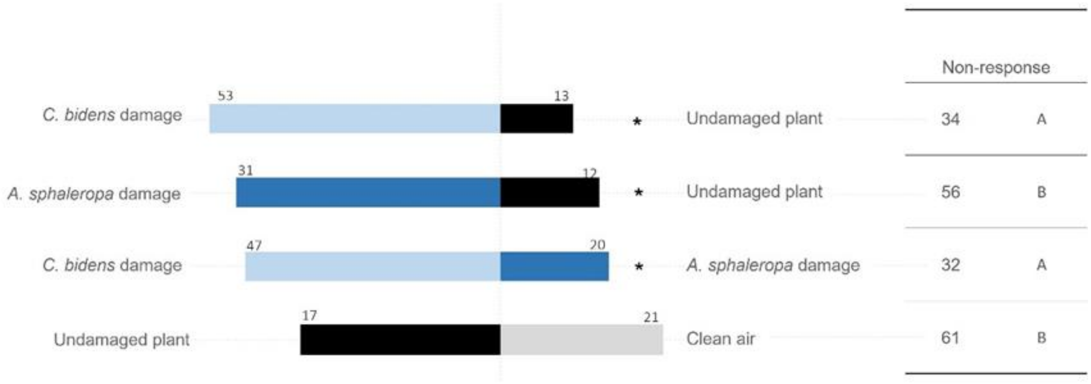

Fig. 5 Preference (first choice) of *Chrysoperla externa* larvae in olfactometer bioassays contrasting odors from pear plants attacked either by *Cacopsylla bidens* or *Argyrotaenia sphaleropa*, compared with undamaged plants. Each bioassay was repeated until reaching N = 40 responsive insects. All numbers indicate percentages, including non-responsive insects to complete 100% of the replicates. Asterisks show significant differences in the exact binomial test for the first arm choice ($P < 0,05$). Different letters under non-responsive insects indicate significant differences of response / no response (GLMbinomial, *Tukey* $P < 0.05$)

When *C. externa* larvae were offered odors from undamaged plants or clean air, they showed no preference (p = 0.5) (Fig. 5). The presence of psylla-damaged plants resulted in lower rates of no-response in the bioassays (Fig. 5) and a faster response in choosing one olfactometer arm (p < 0.05) (Fig. 6).

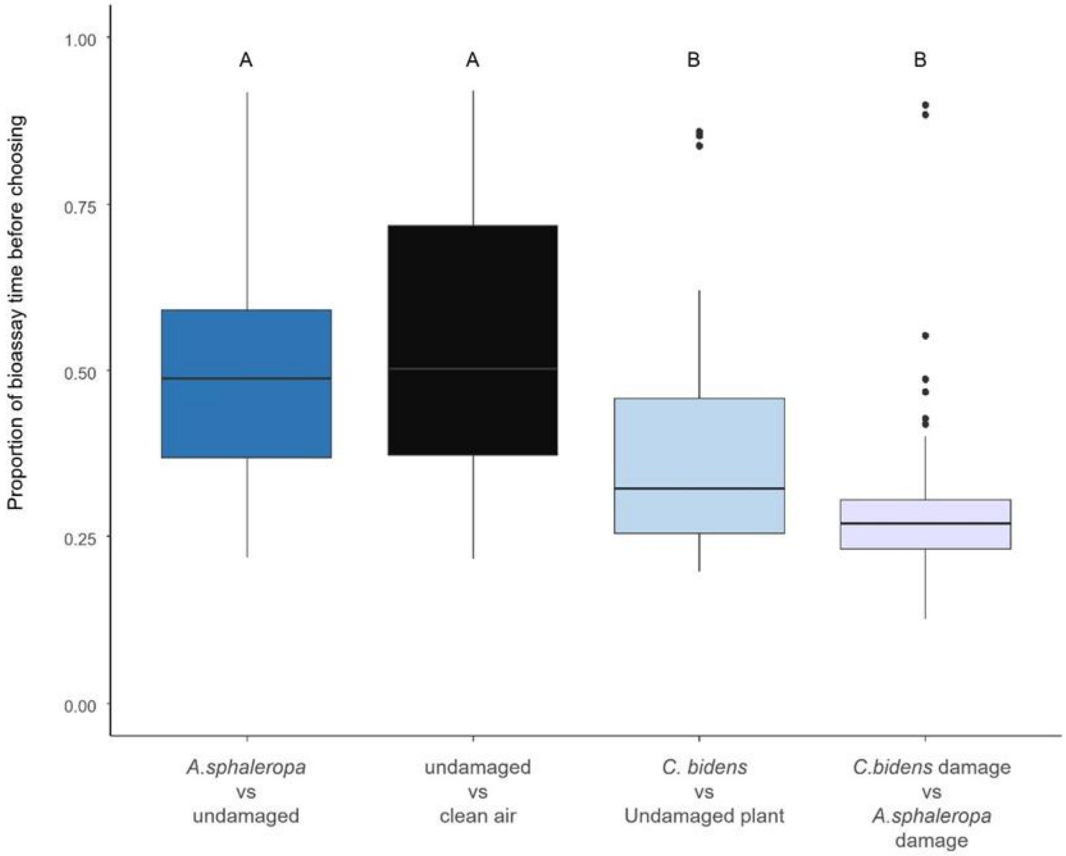

Fig. 6 Boxplots representing the percentage of bioassay time spent by *C. externa* larvae in the olfactometer non-choice zone, before their first arm choice. Different letters indicate statistical differences among bioassays (GLMquasibinomial, *Tukey P* < 0.05)

5.6. DISCUSSION

Our research demonstrates that pear plants modify their volatile profiles in response to herbivore attack. These induced volatiles likely explain the behavioral response of predatory *C. externa* larvae, which are not only attracted to herbivore-damaged plants, but also show differential attraction to odors from psylla-damaged plants to which more predatory larvae respond and they do so more rapidly.

As shown by multivariate and ordination analyses of the volatile blends, undamaged pear plants emit odors that differ from those of plants damaged by either psylla adults or leafroller larvae. Delving into the specific volatile compounds that may explain these differences, the only compound that showed a significant increase in the blend after damage by both herbivores was the sesquiterpene caryophyllene. This compound has been reported as induced in several plants attacked by chewing herbivores, miners and sap-phloem feeders (Ayelo et al. 2021b; Conti et al. 2008; El-Sayed et al. 2018; Kelly et al. 2014; Peterson et al. 2022; Silva et al. 2017; Yang et al. 2021). In addition, caryophyllene has been shown to attract various natural enemies (Jayanthi et al. 2021; Riffel et al. 2021; Zhang et al. 2020). Although this compound is usually regarded as beneficial to the plant, it has been reported as an attractant to *Cacopsylla picta* toward plants affected by the phytoplasma *Candidatus phytoplasma mali*, thereby potentially contributing to the spread of the pathogen (Mayer 2008a, b and 2011). In our study system, caryophyllene represented a very small contribution to the volatile blends, not surpassing 1% of the volatiles in both undamaged and damaged plants.

Most damage-induced volatiles in pear plants were different in plants damaged by either herbivore. Plants damaged by *C. bidens* emitted blends enriched in aliphatic aldehydes, a result in line with previous studies (Scutareanu et al. 1997). Nonanal and decanal were the predominant induced compounds in psylla-damaged plants, becoming major components of the blend. As reported by several authors, these aldehydes are induced by herbivory in several plants and are attractive to parasitoids and predators (Badra et al. 2021; Birkett et al. 2003; Dicke 1999; Du et al. 2022; Rott et al. 2005; Yu et al. 2008). These induced aldehydes may also interact directly with herbivores; they can either be attractive in association with the search for oviposition sites (Borrero-Echeverry et al. 2015; Sarkar et al. 2016), or they may be repellent as observed for *Spodoptera litura*, for which food plant preferences correlate negatively with plant nonanal emissions (Du et al. 2022). An increase in aldehyde emissions in psylla-damaged plants may also influence plant-plant communication and prime the activation of pathogen defenses in neighboring plants (Brambilla et al. 2022; Yi et al. 2009, 2010). Taking into account that *Cacopsylla* species are important phytoplasma vectors, it is conceivable that psylla-induced volatiles such as aliphatic aldehydes may both attract natural enemies and elicit a defensive response in nearby plants (Görg et al. 2021; Gross et al. 2019; Rid et al. 2016).

In the case of pear plants damaged by the leafroller *A. sphaleropa*, the homoterpene (*E*)-4,8-dimethylnona-1,3,7- triene (DMNT) and the aromatic benzeneacetonitrile were the most relevant induced volatiles. Benzeneacetonitrile increased from negligible amounts to become the major volatile component emitted by leafroller-damaged plants. This compound has been reported in the odor blend of melons (Majithia et al. 2021) and as part of the induced response of hybrid aspen after exposure to odors from damaged neighboring branches (Li and Blande 2017), as well as of silver birch under direct herbivory (Koski et al. 2015). The role of benzeneacetonitrile as an attractant of natural enemies has not yet been determined, requiring more bioassays specifically directed to this end.

The homoterpene DMNT, in turn, has been reported in several systems as an herbivore-induced plant volatile (Arimura et al. 2000; Birkett et al. 2003; Degenhardt and Gershenzon 2000; Takabayashi et al. 1994), including both leaf chewers and sap feeders. Several studies have demonstrated that this compound serves as a cue for natural enemies, as an activator of plant defenses and as a deterrent for different herbivores (Arimura et al. 2000; Ayelo et al. 2021b; Borrero-Echeverry et al. 2015; Dicke 1994; James 2003; Takabayashi et al. 1994). Recent research has revealed its role in luring different lepidopteran pests, to the point that traps have been tested with the inclusion of this compound to enhance lepidopteran catches (Knight et al. 2011, 2019; Preti et al. 2021). This compound was not induced by *C. bidens* damage, a result that is in line with previous studies in pear plants (Scutareanu et al. 1997). Another compound that showed a significant increase in the emission from leafroller-damaged plants was (*Z*)-β-ocimene. This compound is well known to mediate the attraction of natural enemies of various herbivores, playing an important role in the indirect defense of plants (Cascone et al. 2015; Farré-Armengol et al. 2017; Mohammed et al. 2020). Several studies have shown that this compound plays a primary role in the communication between plants, leading to an increase in the synthesis of methyl jasmonate in plant tissues and providing greater resistance against pests and diseases (Arimura et al. 2012; Godard et al. 2008; Howe and Jander 2008; Muroi et al. 2011).

Green leaf volatiles showed a significant decrease in blend proportions in leafroller-damaged plants, and a similar trend in psylla-damaged plants. These compounds are expected to increase as a result of herbivore damage, and its activity in eliciting predator responses has been demonstrated in several systems (Dudareva et al. 2006; Gatehouse 2002; Moayeri et al. 2007; Takabayashi and Shiojiri 2019; Van Poecke et al. 2001; Weissbecker et al. 1999; Yu et al. 2008). This result may be explained by the time-window used in our experiments; GLVs are the first compounds to be released when a plant is attacked, while the other compounds are induced only after 24 h (Dudareva et al. 2006; Heil 2008; Pichersky et al. 2006; Turlings et al. 1998). In our study, VOC collections were carried out between 24 and 48 h post-damage, a period in which GLVs most likely decrease their prevalence in the volatile blend from damaged plants (Heil 2008; Turlings et al. 1998).

Our olfactometer bioassays showed that odors emitted by pear plants attacked by psylla adults or leafroller larvae are attractive to green lacewing *C. externa* larvae. These odors were contrasted with those from undamaged plants, so even though no bioassays were performed with pure compound mixtures, it seems reasonable to assume that the behavioral responses of *C. externa* larvae are due to induced volatiles emitted by the damaged plants. The lacewings larvae used in our study were reared on lepidopteran eggs and artificial diet based on chicken liver, with no prior contact with plant volatiles, therefore discarding the possibility of associative learning by previous experience (de Oliveira et al. 2019; Drukker et al. 2000). Since leafroller larvae are not regarded as a common prey consumed by *C. externa*, our initial hypothesis was that lacewing larvae would not respond to the volatiles induced by leafroller damage. This was contradicted by our results, because lacewings larvae were differentially attracted to leafroller-damaged plants when contrasted with undamaged plants. It is possible that induced compounds that are common to both herbivores explain this result. The only such compound was caryophyllene, a compound reported to attract chrysopids when used in baited traps (Flint et al. 1979). Caryophyllene, however, was found in minute amounts, particularly in leafroller-damaged plants, so it seems unlikely that it played a role in our bioassay results. Phenylacetaldehyde has also been shown to attract chrysopids (Tóth et al. 2006, 2009), but in our study was only slightly and not significantly increased in leafroller-damaged plants.

Even though a clear attraction of *C. externa* was observed towards volatiles induced by *A. sphaleropa* against undamaged plants, when contrasted with psylla-damaged plants, *C. externa* larvae clearly preferred the latter. Furthermore, odors from psylla-damaged plants resulted in faster response times and a lower rate of non-responsive chrysopid larvae. Such reduction in response times to locate the prey's habitat plays a primary role in the foraging efficiency of natural enemies, and consequently in their fitness, since the energy and predation risks associated with searching are reduced (Bell 1990; Mills and Heimpel 2018). Our previous studies have shown that *C. externa* readily preys on *C. bidens*. (Valle et al. 2022), and our current results add further evidence that *C. bidens* induces volatiles in pear plants that promote the predator's activity. To our knowledge, no data are available on the response of chrysopid larvae to plant aliphatic aldehydes, so bioassays with the specific psylla-induced aldehyde volatiles are warranted to continue these studies.

While other prey cues may be relevant for *C. externa* larvae, possibly in a shorter range, our results support the notion that *C. externa* larvae exploit HIPV cues to locate pear plants on which potential prey are present, and that they do so with some degree of prey specificity. These results provide fundamental information towards the development of semiochemicals as tools to implement a biological control plan of *C. bidens*. Challenges, however, are to be expected, such as associative learning with no reward, as has been reported for several natural enemies (de Oliveira et al. 2019; Drukker et al. 2000). It is essential to deepen into behavioral studies of both adult and immature chrysopids, study alternative preys or food reward options such as nectar or pollen, in order to move towards an "attract and reward" strategy combining attraction with HIPVs with habitat manipulation to ensure their sustained permanence in the orchards (Pappas et al. 2017; Simpson et al. 2011). The results reported here represent a starting point to develop sustainable and agroecological strategies for the control of psylla in pear orchards.

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6. CONSIDERACIONES FINALES

La posibilidad de desarrollar un plan de control biológico exitoso impone una serie de desafíos que conllevan la necesidad de profundizar los conocimientos actuales de ecología nutricional, de la interacción trófica y de los comportamientos que están en la base de estas interacciones. Este hecho se hará aún más relevante en el momento en que se busque hacer énfasis en un manejo agroecológico de plagas que permita generar un agroecosistema resiliente, basándose en un control biológico de conservación que permita magnificar los servicios ecosistémicos naturalmente presentes (Altieri y Nicholls, 2000, Brzozowski y Mazourek, 2018).

El presente trabajo se enfocó en profundizar los conocimientos necesarios para poder implementar un plan de control biológico exitoso de *C. bidens* en peral. Los resultados obtenidos demuestran que, en nuestro país, en los montes productivos que se basan en un manejo convencional de plagas hay una cierta biodiversidad de enemigos naturales. En la copa de los árboles, los coccinélidos y neurópteros, son los grupos mayoritarios.

Los predadores de *C. bidens* son controladores biológicos ineficientes como consecuencia de su fluctuación poblacional, ya que hay un aumento poblacional de estos en asincronía respecto a la plaga. Al fin del invierno las poblaciones son bajas y *Harmonia axyridis* es el único entomofago que muestra una cierta abundancia en los meses de septiembre y octubre. Es recién a partir de noviembre que el *pool* de predadores incrementa. Por lo contrario, la plaga en estudio comienza a aumentar sus poblaciones a partir de septiembre y completa la primera generación en el mes de octubre, período en el cual el único enemigo natural presente con una cierta abundancia es *H. axyridis,* de esta manera logra completar sus primeras dos generaciones en condiciones de baja presión de depredación.

Al analizar el contenido estomacal de los entomófagos recolectados*, Chysoperla externa* es el predador que con mayor frecuencia se alimenta de *C. bidens*, seguido por los otros dos neurópteros, aún no identificados, y por *H. axyridis* y *C. sanguinea. A. bipunctata*, los míridos y los sírfidos no se alimentan de la plaga. La dinámica de depredación a lo largo de las generaciones de *C. bidens* muestra también una cierta asincronía ya que durante la primera y la segunda generación de *C. bidens, C. sanguinea, H. axyridis y Hemerobiidae sp.* son los únicos predadores que se alimentan de la plaga, aunque la abundancia de estas tres especies es extremadamente baja. Recién en la tercera generación de *C. bidens* comienzan a estar presentes los neurópteros aumentando significativamente sus abundancias y sus actividades de alimentación, particularmente la de *C. externa.*

Para lograr un control eficiente una de las posibilidades es anticipar la llegada de los enemigos naturales, para lograr un control sobre las primeras generaciones de la plaga. Una de las estrategias posibles se basa en utilizar las claves químicas que emite la planta en respuesta al ataque por parte de las plagas. Por este motivo, la segunda parte de esta investigación centró su foco en elucidar los volátiles emitidos por la planta de peral cuando es atacada por *C. bidens*, y a su vez determinar si hay un efecto en el patrón de emisión según del herbívoro que se está alimentando, testeando la respuesta de la planta de peral luego de ser atacada por un insecto masticador (*A. sphaleropa*). Los resultados demuestran que el patrón de volátiles emitido por herbivoría de *C. bidens* y *A. sphaleropa* es diferente, con un claro aumento de aldehídos alifáticos en el caso de daño de *C. bidens*, y un aumento en compuestos aromáticos y homoterpenos en el caso del daño por larvas de *A. sphaleropa*. A su vez, la emisión de volátiles se incrementa en su totalidad respecto a la planta sana, cuando el daño es causado por cualquiera de los dos herbívoros.

El cambio en el perfil de volátiles se asocia a una respuesta comportamental sobre el principal predador de *C. bidens*. Las larvas de *C. externa*, son atraídas diferencialmente por los estímulos odoríferos derivados de plantas que sufrieron el ataque de *C. bidens,* ya sea cuando se compara con plantas sanas, como con plantas dañadas por *A. sphaleropa*. La respuesta en presencia de volátiles emitidos por plantas dañadas por *C. bidens* es más consistente, dado que es menor el número de larvas que no responden. Asimismo, el tiempo de respuesta es menor, permitiendo concluir que estas claves son uno de los insumos que el depredador puede estar usando para ubicar sus presas.

Con la síntesis de los volátiles emitidos por las plantas se podrían desarrollar atrayentes para los controladores naturales de *C. bidens.* Con su uso a nivel comercial la colonización sería más temprana en la temporada y de esta manera habría cierto control sobre las primeras generaciones de la plaga. De todos modos, es necesario tomar en cuenta el agroecosistema en su conjunto y el efecto que el aumento del gremio de predadores pueda tener, tanto sobre la plaga en estudio como sobre otras plagas y, particularmente, en la eventual predación intragremio que podría llevar a una disrupción del control.

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