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Efectos de insecticidas químicos sobre dos enemigos naturales del psílido del tomate *Bactericera cockerelli* (Homoptera: Triozidae), *Tamarixia triozae* (Hymenoptera: Eulophidae) y *Engytatus varians* (Hemiptera: Miridae)

Tesis

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Resumen general

El ectoparasitoide *Tamarixia triozae* (Hymenoptera: Eulophidae) y el depredador *Engytatus varians* (Hemiptera: Miridae) son dos enemigos naturales (EN) del psílido del tomate, *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae), una de las plagas más destructivas de diversos cultivos de solanáceas en México. Las poblaciones de *B. cockerelli* se combaten con insecticidas de distintos grupos toxicológicos que pueden afectar a los EN y comprometer su desempeño. El objetivo de este trabajo fue conocer los efectos letales y subletales de los insecticidas sobre estos EN. En la primera parte de este estudio se evaluó el parasitismo, alimentación y los efectos transgeneracionales de las hembras de *T. triozae* expuestas, en etapa de huevo, larva y pupa, a aceite refinado de soya, imidacloprid y abamectina. Se usaron tres concentraciones de cada compuesto: concentración mínima registrada en campo (CMiRC), la mitad de la CMiRC (½CMiRC) y la concentración letal media (CL_{50}) para ninfas de cuarto ínstar de *B. cockerelli*, el cual es el ínstar más preferido por el parasitoide. El parasitoide tuvo acceso a una mezcla de ínstares del huésped (2do, 3ro, 4to y 5to) para parasitismo y alimentación. En general, el parasismo fue significativamente mayor sobre las ninfas de 4to ínstar (42-96%), seguido por las de 5to (0-81%) y 3ro (6-59%). No se registró parasitismo sobre las ninfas de 2do ínstar. Las hembras de *T. triozae* consumieron más ninfas de 2do ínstar (11-70%) de *B. cockerelli*, seguido por las de 3ro (12-50%), 4to (0-22%) y 5to ínstar (0-17%). Ninguno de los insecticidas modificó la proporción sexual en la generación F_2. En la segunda parte de este trabajo, se evaluó la degradación de spinosad, flufenoxuron, imidacloprid y dimetoato, cuando se asperjaron sobre plantas de *Solanum lycopersicum* L. (tomate) en invernadero, así como la residualidad de estos compuestos sobre los adultos de *E. varians*. Para cada compuesto se utilizaron dos concentraciones, a) el 100% de la concentración máxima recomendada en campo (CMáxRC) (720, 120, 1400 y 1600 mg de ingrediente activo [i.a.]/L, respectivamente) y b) el 50% de la CMáxRC (360, 60, 700 y 800 mg i.a./L, respectivamente). A las 6 h, 10, 20, 30 y 40 días después de la aplicación se colectaron foliolos de tomate para cuantificar los residuos de los insecticidas, mientras la toxicidad sobre *E. varians* se determinó exponiendo a los adultos sobre hojas de las plantas de tomate. A 6 h después de las aspersiones al 100% de la CMáxRC, las concentraciones fueron 73.3 μg/g para spinosin A, 59.1 μg/g para spinosin D, 9.2 μg/g para flufenoxuron, 31.7 μg/g para imidacloprid y 71.4 μg/g para dimetoato. Y fueron 60.0, 45.8, 7.3, 28.3 y 60.0 μg/g, respectivamente, cuando se trataron con el 50% de la CMáxRC. Después de 40 días, cuando se aplicó el 100% de la CMáxRC, las concentraciones de las spinosinas A y D, flufenoxuron y dimetoato fueron aproximadamente la mitad de la inicial. El tiempo medio de vida (TMV) de spinosad, flufenoxuron y dimetoato estuvo entre 34-40 días. Sin embargo, el TMV para imidacloprid fue de 112 días. La mortalidad causada por imidacloprid y dimetoato sobre *E. varians* fue 100% a los 10 días, y disminuyó a alrededor de 30% a los 40 días post-aplicación. En dimetoato e imidacloprid, la mortalidad de *E. varians* fue proporcional a la concentración de los residuos. Flufenoxuron no causó mortalidad durante el experimento, mientras que spinosad causó menos de 10% de mortalidad durante los primeros 10 días. La última parte de este trabajo consistió en determinar la mortalidad de adultos de *E. varians*, cuando se expusieron a foliolos de tomate tratados con tres concentraciones (CMiRC, ½CMiRC y CL_{50} para ninfas de cuarto ínstar de *B. cockerelli*) de abamectina, imidacloprid, bifentrina y flufenoxuron. La ½CMiRC y la CMiRC de bifentrina, abamectina e imidacloprid causaron entre 32-82, 30-98 y 44-100% de mortalidad sobre *E. varians* a las 24, 48 y 72 h post-tratamiento, respectivamente. La CL_{50} de estos tres compuestos causó entre 2-18% de mortalidad. Flufenoxuron no afectó a los adultos de este depredador. Por otra parte, también se evaluaron diferentes parámetros biológicos de las hembras de *E. varians* sobrevivientes a los tratamientos con estos cuatro insecticidas. La CMiRC de bifentrina y la CL_{50} de abamectina disminuyeron significativamente la longevidad de las hembras de *E. varians*. De igual forma, las hembras de este depredador expuestas a las tres concentraciones de los cuatro insecticidas consumieron significativamente menos huevos de *B. cockerelli*, mientras que la CMiRC de bifentrina y la CL_{50} de abamectina afectaron la depredación cuando se les ofreció una mezcla de ninfas de 2do y 3er ínstar (N_2-N_3) de esta plaga. Abamectina, bifentrina y flufenoxuron disminuyeron la fecundidad de las hembras de *E. varians* y solamente la CL_{50} y la ½CMiRC de este último insecticida retrasaron significativamente el desarrollo ninfal. Se concluye que los efectos de los insecticidas evaluados sobre *T. triozae* o *E. varians* dependieron de las concentraciones. Imdacloprid, abamectina, dimetoato y bifentrina causaron las mortalidades más altas sobre *T. triozae* o *E. varians*; por lo tanto, estos insecticidas deben ser usados con precaución en las áreas donde estos enemigos naturales estén presentes o en áreas donde puedan ser liberados para combatir a esa plaga. En contraste flufenoxuron y spinosad no causaron mortalidad significativa sobre los adultos del depredador *E. varians.* Se recomienda realizar más estudios para evaluar los efectos subletales causados por los insecticidas ensayados en el presente estudio.

Palabras clave: Psílido de la papa, enemigos naturales, insecticidas, efectos letales y subletales, manejo integrado de plagas.

General Abstract

The ectoparasitoid *Tamarixia triozae* (Hymenoptera: Eulophidae) and the predator *Engytatus varians* (Hemiptera: Miridae) are two important natural enemies (NE) of the tomato psyllid *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae), one of the most detrimental pests of several solanaceous crops in Mexico. The *B. cockerelli* populations are controlled with insecticides of different toxicological groups that can affect NE and compromise their performance. The objective of this work was to know the lethal and sublethal effects of insecticides on these NE. In the first part of this study, the parasitism, host feeding, and transgenerational effects of *T. triozae* females exposed, in egg, larval, and pupal stages, to the insecticides refined soybean oil, imidacloprid, and abamectin were evaluated. Three concentrations of each compound were used: minimum field-registered concentration (MiFRC), one-half the MiFRC (½MiFRC), and LC_{50} for *B. cockerelli* fourth-instars, the most preferred stage by the parasitoid. The parasitoid had access to a mixture of host instars (2nd, 3rd, 4th, and 5th) for parasitism and feeding. In general, the parasitism was significantly higher on 4th instars (42-96%), followed by 5th (0-81%) and 3rd instars (6-59%) of the host, across all treatments. No parasitism on 2nd instars was observed. *Tamarixia triozae* females consumed more *B. cockerelli* 2nd instars (11-70%), followed by 3rd (12-50%), 4th (0-22%), and 5th instars (0-17%). Any insecticide modified the sex ratio in the F_2 generation. In the second part of this study, the degradation of spinosad, flufenoxuron, imidacloprid, and dimethoate, sprayed on tomato plants (*Solanum lycopersicum* L.) in the greenhouse, as well as the residuality of these compounds on *E. varians* adults was evaluated. For each compound two concentrations, a) 100% of the maximum field-registered concentrations (MaxFRC) (720, 120, 1400 y 1600 mg of active ingredient [i.a.]/L, respectively) and b) 50% of the MaxFRC (360, 60, 700 y 800 mg i.a./L, respectively), were used. At 6 h, 10, 20, 30, and 40 days post-application, tomato leaflets were collected to quantify the residues of the insecticides, while residues toxicity on *E. varians* was determined by exposing adults of this predator on leaves of the tomato plants. At 6 h after spraying at 100% MaxFRC were 73.3 µg/g spinosyn A, 59.1 µg/g spinosyn D, 9.2 µg/g flufenoxuron, 31.7 µg/g imidacloprid, and 71.4 µg/g dimethoate; and 60.0, 45.8, 7.3, 28.3, and 60.0 µg/g, respectively when were treated with 50% MaxFRC. After 40 days, when applied 100% MaxFRC, the concentrations of spinosyns A and D, flufenoxuron, and dimethoate were about half the initial ones. The estimated half-life (DT_{50}) of spinosad, flufenoxuron, and dimethoate was between 34-40 days. However, the DT_{50} for imidacloprid was by 112 days. Mortality caused by imidacloprid and dimethoate on *E. varians* was 100% up to 10 days and decreased to around 30% after 40 days post-application. In dimethoate and imidacloprid, the mortality of *E. varians* was proportional to residue concentration. Flufenoxuron no caused mortality during the experiment, while spinosad caused less than 10% of mortality during the first 10 days. The last part of this work consisted in determining the adult mortality of *E. varians*, exposed on tomato leaflets treated with three concentrations (MiFRC, ½MiFRC, and CL_{50} for *B. cockerelli* fourth-instars) of abamectin, imidacloprid, bifenthrin, and flufenoxuron. The ½MiFRC and MiFRC of bifenthrin, abamectin, and imidacloprid caused between 32-82, 30-98, and 44-100% of mortality on *E. varians* at 24, 48, and 72 h post-treatment, respectively. The LC_{50} of all these three compounds caused between 2-18% of mortality. Flufenoxuron did not affect the adults of this predator. On the other hand, different biological parameters of *E. varians* females surviving to the treatments with these four insecticides were also evaluated. The MiFRC of bifenthrin and the LC_{50} of abamectin, significantly decreased the longevity of *E. varians* females. Likewise, females of this predator exposed to the three concentrations of the four insecticides significantly consumed less *B. cockerelli* eggs, while than the MiFRC of bifenthrin and the LC_{50} of abamectin affected the predation when a mixture of 2nd and 3rd instar (N_2-N_3) of this pest was offered. Abamectin, bifenthrin, and flufenoxuron decreased the fecundity of the *E. varians*, and only the LC_{50} and ½MiFRC of this last insecticide significantly delayed the nymphal development. In conclusion, the effects of the insecticides evaluated on *T. triozae* and *E. varians* depended on concentrations. Imidacloprid, abamectin, dimethoate, and bifenthrin caused the highest mortalities on *T. triozae* or *E. varians*; therefore, these insecticides should be used with caution in areas where these NE are present or in areas where they can be released to combat that pest. In contrast, flufenoxuron and spinosad did not cause significant mortality on *E. varians*. Further studies are recommended to evaluate the sublethal effects caused by the insecticides tested in the present study.

Keywords: Potato psyllid, natural enemies, insecticides, lethal and sublethal effects, integrated pest management.

1. INTRODUCCIÓN GENERAL

El ectoparasitoide *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) se registró por primez vez en México en 2002, parasitando al psílido del tomate, *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae), en Michoacán (Lomelí-Flores y Bueno 2002). Por otra parte, Martínez et al. (2014) reportaron a la chinche zoofitófaga *Engytatus varians* (Distant) (Hemiptera: Miridae) alimentándose sobre ninfas de esta misma plaga en el municipio de Tarímbaro, Michoacán. *B. cockerelli* es una de las plagas más importantes de los cultivos de *Solanum lycopersicum* L. (tomate), *Solanum tuberosum* L. (papa) y *Capsicum annuum* L. (chile) en los Estados Unidos de América (EUA), México, América Central y Nueva Zelanda (Liefting et al. 2009; Munyaneza et al. 2007; Teulon et al. 2009). Este insecto trasmite a la bacteria Candidatus *Liberibacter solanacearum*, la cual se asocia con la enfermedad conocida comúnmente como zebra chip (ZCh) en papa (Munyaneza et al. 2007), aunque también se ha reportado en tomate (Munyaneza et al. 2009a) y chile (Munyaneza et al. 2009b). A la fecha, se han realizado diversos trabajos para conocer la biología del parasitoide *T. triozae* (Morales et al. 2013; Cerón-González et al. 2014, Rojas et al. 2015; Yang et al. 2015; Vega-Chávez et al. 2016; Hernández-Moreno et al. 2017) y la del depredador zoofitófago *E. varians* (Pineda et al. 2016; Dimas-Mora 2017; Esquivel-Ayala 2018; Palma-Castillo et al. 2019). Por otra parte, también se han realizao estuidos para demostrar la capacidad de *T. triozae* (Morales et al. 2013; Ramírez-Ahuja et al. 2017; Calvo et al. 2018a) y *E. varians* (Hernández-Quintero 2015; Velázquez-Rodríguez 2015; Mena-Mociño 2016; Pérez-Aguilar et al. 2019) como agentes potenciales de control biológico de *B. cockerelli*.

Además del control cultural, los insecticidas son una de las principales herramientas que se utilizan para reducir las poblaciones de *B. cockerelli* en el mundo. Algunos de los grupos toxicológicos de mayor uso para el control de esta plaga incluyen a las avermectinas, neonicotinoides, piretroides, fosforados, espinosinas y reguladores de crecimiento, entre otros (Cerna et al. 2013; Dávila-Medina et al. 2012; Garzón et al. 2007; Vega-Gutiérrez et al. 2008). Este tipo de control no es satisfactorio debido a que *B. cockerelli* ha desarrollado resistencia hacia algunos de los insecticidas convencionales (Cerna et al. 2013, 2015; Dávila-Medina et al. 2012). Dentro del concepto de manejo integrado de plagas, el uso de varias tácticas incluído el control biológico y el químico, por ejemplo el uso de *T. triozae* y *E.*

varians con insecticidas de baja residualidad, podría ser una alternativa para reducir las poblaciones de *B. cockerelli*.

Existe una fuente importante de trabajos internacionales que han demostrado que los insecticidas afectan negativamente diversos parámetros biológicos de los enemigos naturales (Desneux et al. 2007; Luna-Cruz et al. 2011, 2015; Morales et al. 2018; Pérez-Aguilar et al. 2018). La mortalidad (efecto letal) causada por los insecticidas químicos es el parámetro que más se ha estudiado, mientras que otros efectos indirectos (efectos subletales) tales como el efecto sobre la emergencia de adultos, capacidad de parasitismo, alimentación, proporción de sexos, apareamiento, preoviposición, oviposición, depredación y longevidad de los individuos que sobreviven a exposiciones de insecticidas se han subestimado (Desneux et al. 2007; Stark et al. 2007). Estos efectos pueden afectar el éxito de un agente de control biológico. Además, la implementación del uso de más de un enemigo natural podría aumentar las posibilidades de control de un insecto plaga y como consecuencia reducir el uso de plaguicidas (Velasco-Hernández et al. 2013; Remírez-Ahuja et al. 2017). De igual forma, conociendo mejor la compatibilidad de ciertos insecticidas menos residuales, y su efecto en los enemigos naturales del psílido *B. cockerelli* se podrían diseñar mejores propuestas de manejo para disminuir los daños de esta plaga.

Es importante mencionar que en estudios de laboratorio y campo, los insecticidas denominados de baja residualidad también pueden causar efectos letales y subletales sobre los enemigos naturales tanto en sus estados inmaduros como en el adulto (los benéficos pueden incluir polinizadores y descomponedores), como se ha reportado para *T. triozae* (Luna et al. 2011, 2015; Liu et al. 2012; Martínez et al. 2015; Morales et al. 2018) y *E. varians* (Bruno-Pérez 2018; Pérez-Aguilar et al. 2018; Morales et al. 2019). Por lo tanto, con el fin de diseñar un programa de manejo integrado para el psílido del tomate, es necesario realizar más estudios toxicológicos para conocer mejor los efectos letales y subletales que causan sobre los enemigos naturales, y determinar cómo afectan el desempeño de estos agentes de control biológico.

2. ANTECEDENTES

2.1. Problemas fitosanitarios en el cultivo de tomate

Los sistemas de producción, tanto en campo abierto como en ambientes protegidos, se encuentran amenazados por diversas especies de artrópodos plaga. Las principales plagas que se encuentra en el cultivo de tomate pueden causar daños directos o indirectos. Entre estas plagas se encuentra el minador de la hoja, *Liriomyza* spp. (Frick) (Diptera: Agromyzidae) (Palacios et al. 2015); gusano soldado, *Spodoptera exigua* (Hübner); gusano falso medidor, *Trichoplusia ni* (Hübner), y gusano del fruto, *Heliotis zea* (Boddie) (Lepidoptera: Noctuidae) (Ortiz-García y Barreiro-García 1994); mosca blanca, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae); trips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae); pulgón verde, *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) (Cabello et al. 1990) y el pulgón saltador o psílido del tomate, *B. cockerelli* (Munyaneza et al. 2007, 2012).

2.2. *Bactericera cockerelli* (Sulc.)

En 1909 T. D. Cockerell colectó por primera vez especímenes de *B. cockerelli* en el estado de Colorado, EUA. En este mismo año, Sulcer (1909) realizó la descripción formal y le asignó el nombre de *Trioza cockerelli,* pero más tarde fue reconocido como *Paratrioza cockerelli.* Actualmente, *B. cockerelli* se ha publicado en la familia Triozidae, superfamilia Psylloidea y es conocida comúnmente como pulgón saltador, psílido de la papa, psílido del tomate, salerillo o simplemente paratrioza (Bujanos et al. 2005). Esta especie se encuentra ampliamente distribuida en EUA, México, Guatemala, Honduras (Munyaneza 2012) y Nueva Zelanda (Liefting et al. 2008; Teulon et al. 2009). En México, *B. cockerelli* se encuentra distribuida en los estados de Sinaloa, Durango, Coahuila, Nuevo León, Guanajuato, Michoacán, Puebla y Tamaulipas, entre otros (Cerna et al. 2013, 2015; Rubio et al. 2006; Vega-Gutiérrez et al. 2008).

2.2.1. Tipos de daños y métodos de control

En México, a partir de los años 70´s, *B. cockerelli* se convirtió en una plaga primaria de los cultivos de papa, chile y tomate (Vega-Gutiérrez et al. 2008). Este insecto ocasiona daños directos al succionar la savia de las plantas (Bujanos et al. 2005; EPPO 2013) e indirecto al transmitir el patógeno que causa la enfermedad conocida como zebra chip (ZCh) en papa (Butler y Trumble 2012a; EPPO 2013; Dávila-Medina et al. 2012; Munyaneza 2012). Los métodos que tradicionalmente se utilizan para el manejo de las poblaciones de *B. cockerelli* incluyen al control cultural, químico y biológico. El control cultural incluye la destrucción de plantas hospederas de la plaga antes de las nuevas plantaciones, y en los márgenes del cultivo y campos adyacentes durante la temporada de cultivo (Butler y Trumble 2012a; Munyaneza 2012, 2015).

El control químico contra los artrópodos plaga, incluida *B. cockerelli,* es la práctica más frecuente utilizada en los sistemas agrícolas (Global Crop Protection 2017). Sin embargo, las aplicaciones frecuentes de insecticidas han provocado un desequilibrio en el medio ambiente, daños a la salud de los trabajadores y consumidores (Wasi et al. 2013; Özkara et al. 2016) y surgimiento de poblaciones de insectos resistentes (Cerna et al. 2013, 2015; Pardo et al. 2018; Chen et al. 2018). La aplicación de algunos insecticidas neonicotinoides (imidacloprid y thiamethoxam), carbamatos (aldicarb) y fosforados se realiza directamente al suelo debido a que actúan como sistémicos y combaten a los estados de ninfas y adultos de *B. cockerelli* (Bujanos et al. 2005; Garzón et al. 2007).

El control biológico de *B. cockerelli* mediante sus enemigos naturales tales como hongos entomopatógenos (Tamayo-Mejía et al. 2014, 2015) o entomófagos (Rojas et al. 2015, Ramírez-Ahuja et al. 2017, Calvo et al. 2018a, 2018b) ayuda sustancialmente a disminuir el uso excesivo de insecticidas químicos (Bahena 2008; Nicholls 2008).

2.3. Enemigos naturales para el control de *B. cockerelli*

El control biológico es parte del manejo integrado de plagas (MIP), y se refiere al uso de enemigos naturales (patógenos, depredadores y parasitoides) para la disminución de las poblaciones de insectos que causan daños económicos a los cultivos, coadyuvando en el incremento de su productividad y calidad (Messelink et al. 2014; van Lenteren et al. 2018). Las especies de hongos entomopatógenos que se han investigado para el control de *B. cockerelli* se encuentran *Beauveria bassiana* (Balsamo) (Ascomycota: Hyphoreales), *Metarhizium anisopliaeae* (Metchnikiff) (Hypocreales: Clavicipitaceae), *Isaria fumosorosea* ([Wize] A.H. S. Br. & G. Sm. 1957) (Hypocreales: Clavicipitaceae) (Lacey et al. 2011;

Tamayo-Mejía et al. 2014, 2015). Estas tres especies de hongos entomopátogenos han causado hasta 90% de mortalidad sobre huevos y ninfas de *B. cockerelli* en condiciones de campo sobre plantas de papa y chile (Lacey et al. 2011; Tamayo-Mejía et al. 2014).

Los parasitoides primarios de *B. cockerelli* son *Metaphycus psyllidis* (Compere) (Butler y Trumble 2012a, 2012b) y *T. triozae* (Lomelí-Flores y Bueno 2002). En ensayos de elección y no elección *Diaphorencyrtus aligarhensis* (Shafee, Alam, & Agarwal) (Hymenoptera: Encyrtidae) causó 19% de parasitismo sobre ninfas de *B. cockerelli* (Bistline-East et al. 2015). Por otro lado, existen diversas especies de depredadores de *B. cockerelli*, dentro de los cuales se encuentran *Chrysoperla* sp. (Neuroptera: Chrysopidae), *Hippodamia convergens* (Guerin-Meneville), *H. quinquesignata* (Kirby), *H. americana* (Crotch) (Coleoptera: Coccinellidae), *Geocoris decoratus* (Uhler) (Hemiptera: Geocoridae), *Orius tristicolor* (White), *Anthocoris tomentosus* (Pericart) (Hemiptera: Anthocoridae), *Nabis ferus* (L.) (Hemiptera: Nabidae), *Deraeocoris brevis* (Uhler), *E. varians* (Hemiptera: Miridae) y *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) (Butler y Trumble 2012a, 2012b; Martínez et al. 2014). Adicionalmente, a la lista de depredadores se incluyó al menos un par de depredadores zoofitófagos que se pueden alimentar de *B. cockerelli*, estos son *Dicyphus hesperus* (Calvo et al. 2016, 2018) y *Engitatus varians* (Martínez et al. 2014; Pineda et al. 2016; Palma-Castillo et al. 2019; Pérez-Aguilar et al 2019). A continuación se describirán algunos aspectos sobre la biología de *T. triozae* y *E. varians* por ser los enemigos naturales utilizados en el presente estudio.

2.4. *Tamarixia triozae*

Es un ectoparasitoide que se registró por primera vez en el Norte de América en el estado de Colorado, EUA (Jensen 1957; Pletsch 1947). En Jacona de Plancarte, Michoacán, Lomelí-Flores y Bueno (2002) reportaron la presencia de *T. triozae* sobre *B. cockerelli* y, de acuerdo a sus observaciones, lo consideró como un agente promisorio de control biológico para esta plaga. Bravo y López (2007) reportaron hasta 80% de parasitismo de *T. triozae* sobre *B. cockerelli* en cultivos de chile en los valles centrales de Oaxaca, donde no se utilizaron insecticidas. En el sur de Texas, *T. triozae* causó entre 5-20% de parasitismo sobre ninfas de *B. cockerelli* en cultivos de papa a campo abierto (Liu et al. 2012) y entre 20-30% en condiciones de laboratorio en tomate y chile pimiento (Morales et al. 2013). Las hembras de

T. triozae pueden parasitar a las ninfas del tercero, cuarto y quinto estadio de *B. cockerelli*, pero tienen preferencia por las de cuarto y quinto estadio (Morales et al. 2013; Cerón-González et al. 2014; Rojas et al. 2015).

2.4.1. Hábito y ciclo biológico

Tamarixia triozae es un parasitoide sinovigénico; es decir, además de causar la muerte por por parasitismo, también puede alimentarse de la hemolinfa de su huésped para obtener los nutrientes necesarios para el desarrollo de sus huevos, lo que se conoce comúnmente como alimentación sobre el huésped (Chan y Godfray 1993; Kidd y Jervis 1991). Para realizar la oviposición, las hembras de este parasitoide paralizan temporalmente a las ninfas hospederas de *B. cockerelli*, posteriormente colocan sus huevos entre el primer y el segundo par de coxas o entre el segundo o tercer par de coxas (Martínez et al. 2015). Después de su oviposición, la ninfa huésped queda paralizada por un corto tiempo (≈ 1 minuto) y posteriormente continua alimentándose (Martínez et al. 2015).

2.5. *Engytatus varians*

El género *Engytatus* Reuters se distribuye en varias partes del mundo, desde el sur de la región Neártica, Neotropical y diversas islas de la región Oriental (Cassis y Schuh 2012; Martínez et al. 2014). La especie *E. varians* se distribuye ampliamente en el norte (Madden y Chamberlin 1945; Martínez et al. 2014), centro (Maes 1998) y sur (Schuh 1995) de América. En México, Martínez et al. (2014) reportaron por primera vez a *E. varians*, depredando a ninfas del psílido sobre plantas de tomate bajo condiciones de invernadero en El Trébol, municipio de Tarímbaro, Michoacán.

Rosewall y Smith (1930) reportaron a ninfas y adultos de *E. varians* depredando huevos y larvas de los primeros estadios de *Heliotis* spp., así como huevos y larvas de *Heliotis virescens* (F.) y *H. obsoleta* (F.) (Lepidoptera: Noctuidae) en campos de tabaco en Luisiana, EUA. En condiciones naturales, Illingworth (1937) observó la depredación de *E. varians* sobre áfidos del tabaco, pseudocóccidos, así como huevos y larvas de primeros estadios de la mariposa de la col *Pieris brassicae* (L.) (Lepidoptera: Pieridae). En Cuba *E. varians* se reportó alimentándose sobre ninfas de *B. tabaci* en cultivos de papa, tabaco y cucurbitáceas (Castineiras 1995). Estudios realizados en Brasil, demostraron que *E. varians* puede controlar de manera efectiva a *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) sobre plantas de tabaco, ya que tuvo una tasa de depredación de 92 huevos de esta plaga en un periodo de 24 h (Bueno et al. 2013). De igual forma, en condiciones de laboratorio, *E. varians* depredó hasta 90% de ninfas de *B. cockerelli* en plantas de tomate (Martínez et al. 2014; Velázquez-Rodríguez et al. 2015).

2.5.1 Hábito zoofitófago de las chinches de la familia Miridae

La zoofitofagia es un caso especial de omnivoría en las chinches de la familia Miridae ya que algunas especies se alimentan tanto de plantas como de presas que se puedan encontrar en la misma planta hospedera (Gillespie y McGregor 2000).

Los depredadores zoofitófagos son generalmente percibidos como menos eficientes para el mantenimiento de los niveles de plagas ya que al alimentarse de la planta puede reducir el consumo de sus presas (Castañe et al. 2011). Sin embargo, liberaciones en cultivos protegidos de tomate sugieren que los depredadores zoofitófagos son capaces de establecerse y alimentarse exitosamente de los insectos plaga que se encuentren presentes (Calvo et al. 2016, 2018a, 2018b; Esquivel-Ayala 2018; Pérez-Aguilar et al. 2019; Moerkens et al. 2017).

En cultivos de hortalizas, el uso de depredadores zoofitófagos ha estado enfocado principalmente en cuatro especies: *Dicyphus tamaninii* Wagner, *D. hesperus* (Knight), *Macrolophus pygmaeus* (Rambur) y *Nesidiocoris tenuis* (Reuter), todas pertenecientes a la familia Miridae. Estos depredadores generalistas regulan las poblaciones de otros artrópodos de cuerpo blando tales como mosca blanca, áfidos, araña roja, minadores de hojas y trips, entre otros (Shipp y Wang 2006, Urbaneja et al. 2001). La conservación de poblaciones nativas de *N. tenuis, D. tamaninii* y *M. pygmaeus* es una estrategia de manejo de plagas que ha sido usada satisfactoriamente en la región del Mediterráneo (Castañe et al. 2011). En México, desde el año 2014, se han realizado diversos estudios sobre la biología de *E. varians* (Pineda et al. 2016; Dimas-Mora 2017; Esquivel-Ayala 2018; Palma-Castillo et al. 2019) así como de su potencial como depredador hacia ninfas y adultos de *B. cockerelli* (Martínez et al. 2014; Velázquez-Rodríguez et al. 2015; Mena-Mociño 2016; Pérez-Aguilar et al. 2019).

2.6. Insecticidas

Los insecticidas han contribuido sustancialmente al mejoramiento de la productividad agrícola en el mundo. Paralelamente, su uso ha tenido efectos adversos que incluyen el deterioro ecológico y el daño a la salud humana (Özkara et al. 2016; Ndakidemi et al. 2016; García-Gutiérrez y Rodríguez-Meza 2012). Sin embargo, existe una investigación constante por descubrir nuevas moléculas con propiedades insecticidas más amigables con el medio ambiente, que sean más específicos y que tengan baja persistencia y toxicidad hacia organismos no blanco (Singh et al. 2015; Kodandaram et al. 2016; Carvalho 2017).

Aceites: Son productos que incluyen aceites destilados de petróleo o aceites minerales, así como también de plantas y animales. Los insecticidas a base de aceites están regulados por la Agencia de Protección del Medio Ambiente (EPA, por sus siglas en inglés) en EE.UU., Comisión Federal para la Protección contra Riesgos Sanitarios (COFEPRIS), Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT) y Secretaría de Agricultura y Desarrollo Rural (SADER) en México. Son insecticidas seguros y no persistentes en el medio ambiente. Los productos a base de aceites causan la muerte de los insectos alterando la función de la membrana celular epidérmica y por la obstrucción de los espiráculos en el intercambio de gases, causando la asfixia (Bográn et al. 2014; Helmy et al. 2012).

Avermectinas: Las avermectinas son productos naturales derivados del proceso de fermentación de la bacteria *Streptomyces avermitilis* (Kim y Goodfellow), un actinomiceto del suelo. Dentro de este grupo se encuentra el insecticida abamectina, una mezcla de dos lactonas macrocíclicas neurotóxicas (avermectina B_{1a} y B_{1b}). Las avermectinas actúan estimulando, en la célula pre-sináptica, la liberación de un neurotransmisor inhibitorio, el ácido gamma amino butírico (GABA), el cual se acopla a los receptores post-sinápticos bloqueando la señal de la transmisión de las uniones neuromuscular, causando parálisis, ¿? cese de alimento y muerte del insecto (Ananiev et al. 2002).

Neonicotinoides: Son productos diseñados a partir de la nicotina. Dentro de este grupo se encuentra el imidacloprid, el cual actúa sobre los receptores nicotínicos que se encuentran en la zona post-sináptica, bloqueando la unión del neurotransmisor acetilcolina que es expulsado de la zona pre-sináptica, causando una parálisis en el insecto que los lleva a la muerte por un descontrol en su sistema nervioso (Zhang et al. 2000). Este compuesto actúa por contacto y es sistémico (Simon-Delso et al. 2015).

Benzoylfenilureas: Son insecticidas reguladores de crecimiento de los insectos (RCI), los cuales interrumpen el proceso de la muda a través de la inhibición de la síntesis de quitina durante la formación de la cutícula, lo que causa una deposición endocuticular anormal en el insecto en sus etapas inmaduras (Merzendorfer 2013; Khater 2011; Kodandaram et al. 2010). Los RCI actúan principalmente por ingestión y contacto (Kodandaram et al. 2010).

Spinosinas: Son insecticidas derivados del proceso de fermentación del actinomiceto del suelo *Saccharopolyspora spinosa* Mertz & Yao (Actinomycetales: Pseudonocardiaceae) (Thompson et al. 2000). Spinosad, compuesto por las spinosinas A (85%) y D (15%), se considera un insecticida con toxicidad relativamente baja para los mamíferos y un perfil ambiental favorable (Thompson et al. 2000; Crouse et al. 2001; Nauen y Bretschneider 2002). Spinosad actúa principalmente a través de la ingestión, pero también tiene cierta actividad de contacto. Este compuesto actúa sobre el sistema nervioso del insecto como modulador alostérico de los receptores nicotínicos de la acetilcolina (nAChR) y también exhibe actividad en la fisiología del receptor del aminoácido γ-butírico (GABA) (Thompson et al. 2000; Biondi et al. 2012).

Organofosforados: Los insecticidas organofosforados son de rápida degradación en el medio ambiente y altamente efectivos en el control de plagas de insectos (Pope 2012; Pérez-Zarate et al. 2016; Wright et al. 2017). La toxicidad de estos insecticidas se inicia mediante la inhibición de la enzima acetilcolinesterasa (AChE) en el sistema nervioso central y periférico (Gunning y Moores 2001; Pope 2012).

2.7. Efecto de los insecticidas sobre los enemigos naturales

El uso conjunto de insecticidas y enemigos naturales en el control de insectos plaga en sistemas agrícolas puede afectar el desempeño biológico de estos seres vivos. Lo anterior debido a las características intrínsecas de los insectos benéficos, las cuales incluyen menor talla (en proporción a la mayor superficie de exposición al plaguicida), mayor movilidad, sistemas enzimáticos diferentes y estrecha dependencia de sus huéspedes (Talebi et al. 2008; Vargas y Ubillo 2001; Singh 2015; Ndakidemi et al. 2016).

En general, la forma de contaminación de los enemigos naturales con los diferentes insecticidas químicos puede llevarse a cabo de las dos formas siguientes: i) Contaminación directa a través de la ingestión o por contacto con sus tarsos, ii) En los estados inmaduros de los parasitoides, y en depredaores, al alimentarse de su huésped o presa contaminada (Desneux et al. 2007; Ndakidemi et al. 2016).

2.7.1. Efectos letales y subletales

En relación a los estudios que se han realizado sobre los efectos letales causados por diferentes insecticidas sobre *T. triozae*, Luna-Cruz et al. (2011) reportaron que abamectina y spinosad fueron altamente tóxicos ya que causaron más de 90% de mortalidad sobre los adultos de este parasitoide. En contraste, estos mismos autores reportaron que azadiractina e imidacloprid causaron bajos niveles de mortalidad (2-8%) sobre los adultos de este parasitoide. En otro estudio, Luna-Cruz et al. (2015) encontraron mortalidades de adultos superiores a 90% para abamectina y spinosad, mientras que azadiractina, imidacloprid, spirotetramat, spiromesifen, pymetrozine y beta-cyfluthrin registraron entre 25 y 50%, a los 13 días. Resultados similares fueron reportados por Martínez et al. (2015) con abamectina (72-100%), imidacloprid (28-58%) y el aceite refinado de soya (5-39%) cuando los adultos de *T. triozae* se colocaron sobre hojas de tomate y pimiento previamente tratadas por aspersión o inmersión. Liu et al. (2012) evaluaron el efecto de 11 insecticidas sobre los adultos de *T. triozae* y observaron que spinetoram, laverage 2.7 (imidacloprid + cyfluthrin), abamectina y tolfenpyrad causaron 100% de mortalidad, mientras que los insecticidas cyantraniliprole, fenpyroximate, novaluron, pymetrozine, spirotetramat, spiromesifen y el aceite de *Chenopodium ambrosioides* nr. *ambrosiodes* provocaron un rango de mortalidad ente 2 y 50%. De igual forma, Morales et al. (2018) registraron una mortalidad variable cuando las etapas inmaduras (huevo, larva y pupa) de *T. triozae* se trataron por inmersión con varias concentraciones de un aceite de soya, imidacloprid y abamectina.

En relación a los efectos subletales, la emergencia de adultos de *T. triozae* fue entre 9-71% y 60-80% cuando se trataron en las etapas de huevo y larva, respectivamente, con un aceite de soya, imidacloprid o abamectina (Morales et al. 2018). Por otro lado, la emergencia de adultos se inhibió completamente cuando las pupas del parasitoide se trataron con imidacloprid (Luna et al. 2011) y abamectina (Martínez et al. 2015). Sin embargo, Morales et al. (2018) reportaron 100 y 83% de emergencia de adultos de *T. triozae* cuando el parasitoide fue tratado en esta misma etapa con imidacloprid y abamectina, respectivamente.

Además, el aceite de soya, abamectina, laverage 27 (imidacloprid+cyfluthrin), imidacloprid y spinetoram afectaron drásticamente la sobrevivencia de hembras y machos de *T. triozae* cuando fueron expuestos en etapa adulta o inmadura (Liu et al. 2012; Morales et al. 2018).

Con respecto a estudios realizados sobre la toxicidad al depredador *E. varians,* Bruno-Pérez (2018), Pérez-Aguilar et al. (2018) y Morales et al. (2019) reportaron que dimetoato, abamectina, imidacloprid, bifentrina, clorfenapir y tiametoxam causaron mortalidades entre 90 y 100% en adultos y ninfas de *E. varians,* teflubenzuron entre 24 y 66% de mortalidad (Pérez-Aguilar et al. 2018), mientras que flufenoxuron no causó ningún efecto (Bruno-Pérez 2018; Morales et al. 2019). Por otro lado, poco se conoce sobre los efectos subletales causados por los inhibidores de la síntesis de quitina sobre el depredador *E. varians*. Al respecto, la longevidad y descendencia de las hembras de este depredador disminuyeron cuando las ninfas de tercer instar se expusieron a residuos de teflubenzuron (Pérez-Aguilar et al. 2018). La depredación causada por *E. varians* hacia una mezcla de ninfas de segundo y tercer instar de *B. cockerelli*, así como la longevidad, descendencia y desarrollo ninfal fueron afectadas cuando los adultos se expusieron a los residuos de 6 h de edad de flufenoxuron (Bruno-Pérez 2018).

Las predicciones sobre la compatibilidad de un insecticida con el control biológico se basan en estudios incompletos debido a que la mayoría se realizan sobre un estado de vida del insecto benéfico (más frecuentemente el adulto). Por ello, se deben realizar estudios más completos que evalúen los efectos letales y subletales para tener claro el impacto que puedan tener estos insecticidas sobre los enemigos naturales.

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3. HIPÓTESIS

Los insecticidas flufenoxuron, imidacloprid, abamectina, bifentrina y aceite de soya causan efectos letales y subletales (fisiológico y en el comportamiento) sobre el parasitoide *T. triozae* y el depredador *E. varians.*

Los residuos de spinosad, flufenoxuron, imidacloprid y dimetoato presentes en hojas de tomate son inocuos para el depredador *E. varians*, bajo condiciones de invernadero.

4. OBJETIVOS

4.1. General

Conocer los efectos letales y subletales causados por insecticidas de diferente modo de acción sobre el parasitoide *T. triozae* y el depredador *E. varians*.

4.2. Particulares

- Determinar los efectos subletales (parasitismo, alimentación y proporción de sexos) causados por el aceite de soya, imidacloprid y abamectina sobre *T. triozae* provenientes de los estados de huevo, larva y pupa tratados con estos insecticidas.

- Evaluar la cinética de degración de spinosad, flufenoxuron, imidacloprid y dimetoato después de su aplicación sobre plantas de tomate en condiciones de invernadero y la evolución de su toxicidad residual sobre los adultos de *E. varians*.

- Determinar los efectos letales (mortalidad) y subletales (longevidad, depredación, fecundidad y tiempo de desarrollo) causados por imidacloprid, abamectina, bifentrina y flufenoxuron sobre el depredador *E. varians*.

Parasitism, host feeding, and transgenerational effects of three insecticides on the eulophid *Tamarixia triozae* when exposed in immature stages

Abstract

The ectoparasitoid *Tamarixia triozae* is a promising biological control agent of the tomato psyllid, *Bactericera cockerelli,* based on its high parasitism rates on different crops. The parasitism, host feeding, and transgenerational effects (in terms of sex ratio) of *T. triozae* females exposed to three insecticides (soybean oil, imidacloprid, and abamectin) as eggs, larvae, and pupae were evaluated when a mixture of second-, third-, fourth-, and fifth-instars of the host *B. cockerelli* was offered. When the effects of three concentrations of these insecticides (minimum field-registered concentration [MiFRC], one-half the MiFRC [½MiFRC], and median lethal concentration [LC_{50}] for *B. cockerelli* fourth-instars) were evaluated, no parasitism of *B. cockerelli* second instars was recorded when parasitoid's females were treated in the three immature stages with any of the insecticides. In contrast, in some cases, parasitism of *T. triozae* females treated as eggs, larvae or pupae with soybean oil and imidacloprid was reduced in third, fourth or fifth instar. Parasitism of *T. triozae* females treated as eggs with LC_{50} of abamectin was reduced in third instars, whereas when the parasitoid was treated both as larvae and pupae, it was reduced in fifth instar of the host. In most cases, the host feeding was reduced in second and third instar of the host *B. cockerelli* when *T. triozae* females were treated as eggs, larvae or pupae. Any insecticide modified the sex ratio in the F2 generation. In conclusion, both parasitism and host feeding were affected by the insecticides depending on the concentration and on the nymphal instar of the host *B. cockerelli* offered.

Key words: *Bactericera cockerelli*; Sublethal effects; Sex ratio; Biological control; Chemical compounds.

Morales S.I., S. Pineda, E. Viñuela, J.I. Figueroa, F. Tamayo, E. Rodríguez-Leyva, A.M. Martínez. Parasitism, host feeding, and transgenerational effects of three insecticides on the eulophi *Tamarixia triozae* when exposed in immture stage. **Sometido a *Ecotoxicolgy*.**

Introduction

The implementation of general principles of integrated pest management (IPM) and the sustainable use of pesticides are now recognized to be necessary in modern agriculture (Ehi-Eromosele et al. 2013). One of the most valuable IPM tactics in many crops is biological control -the use of natural enemies to decrease the density of key pest organisms (van Lenteren 2012; Nafiu et al. 2014)- and in 2015, the international market of biological control agents (invertebrates and microorganisms) was approximately US$ 1.7 billion (Dunham 2015; van Lenteren et al. 2018). Biological control is considered as one of the most environmentally safe and effective pest management methods worldwide (Williams et al. 2013; Nafiu et al. 2014) and its use is promoted through regulations in many countries because it has low impact on human health, greenhouse gases emissions or farm machinery investment, among other factors (Benjamin and Wesseler 2016). However, when the availability of natural enemies is low or they do not offer a complete solution to the challenge some pests pose in agriculture, farmers can postpone its adoption in favor of chemical control (Viñuela 2005; Talebi et al. 2008; Biondi et al. 2012) even though the intensive use of pesticides prevents its success (van Lenteren and Bueno 2003)**.**

Due to its versatility and rapid action against pests, chemical control still remains as the principal tool used by farmers for pest control in agroecosystems (Pérez-González and Sánchez-Peña 2017; Wright et al. 2017; Tang et al. 2018). In spite of pesticide damages to environment and society (public health, livestock and livestock product losses, pest control problems due to the reduction of natural enemies and development of pesticide resistance, crop pollination problems, wildlife losses) and of the governmental expenditures implemented to reduce the environmental and social cost of the application of pesticides (Pimentel 2005; Carvalho 2017), global pesticide production has increased at 11% per year from the 1950s to 2000 (more than 5 million tons) (FAO 2017). Although pesticides are the increasingly applied with techniques and concentrations that aim to avoid harm to non-target organisms (Singh et al. 2015; Carvalho 2017; Mohammed et al. 2018), insect natural enemies are often more affected by chemical pesticide applications than pests (Lee 2000; Stark and Banks 2003; Stark et al. 2014). As result, the use of pesticides can severely disrupt the performance of biological control agents (Viñuela 2005; Biondi et al. 2012; Rafiee-Dastjerdi et al. 2012).

Tamarixia triozae (Burks) (Hymenoptera: Eulophidae) was reported for the first time by Burks (1943) and it was detected for the first time in Mexico in 2002 parasitizing nymphs of the tomato psyllid *Bactericera cockerelli* (Sulcs) (Hemiptera: Triozidae) (Lomelí-Flores and Bueno 2002), the most serious pest of potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), and several other solanaceous crops (Garzón-Tiznado et al. 2009; Camacho-Tapia et al. 2011). *Tamarixia triozae* is considered to be the most important ectoparasitoid of *B. cockerelli* because its parasitism rates on this pest can reach 20% on potato and tomato (Liu et al. 2012) or 80% on pepper in the field (Bravo and López 2007), and around 30% on both tomato and pepper in the laboratory (Morales et al. 2013). This parasitoid is idiobiont and synovigenic (Morales et al. 2013; Rojas et al. 2015) and females feed on the host hemolymph or tissues, furnishing the necessary nutrients for egg production and maturation (Jervis and Kidd 1986; Heimpel and Collier 1996). As such, in mixed-instar experiments, *T. triozae* females ate between 36 and 58% of *B. cockerelli* hosts (Morales et al. 2013; Cerón-Gonzalez et al. 2014).

Different aspects of *T. triozae* biology have been elucidate in the last years: its biology, life history and potential as a biological control agent (Rojas et al. 2015); the influence of host plant on parasitism and host feeding rates (Morales et al. 2013; Cerón-González et al. 2014; Martínez et al. 2015; Hernández-Moreno et al. 2017); and the effects of some insecticides (Luna-Cruz et al. 2011, 2015; Martínez et al. 2015; Morales et al. 2018), or entomopathogenic fungi (Tamayo-Mejía et al. 2015). It is known that several insecticides belonging to different chemicals groups (e.g., neonicotinoids, avermectins, spinosyns, botanicals, pyrethroids, diamides, pyridines, oil-based, and benzoylureas) can cause mortality to *T. triozae* adults through different exposure methods: residual contact with treated glass vials or leaf surfaces and ingestion (Luna-Cruz et al. 2011, 2015; Liu et al. 2012; Martínez et al. 2015). However, the determination of pesticide mortality only does not provide a robust estimate of stress (Guedes et al. 2016) and more long-term effects must be studied because they can also compromise the beneficial performance of natural enemies (Rafiee-Dastjerdi et al. 2012; Sohrabi et al. 2012, 2013; Fernández et al. 2010, 2015). As such, decreases in the parasitism and adult longevity rates have also been reported in *T. triozae* when adults ingested the insecticides (Liu et al. 2012). In addition, it is known that pesticides can also cause transgenerational effects and affect developmental stages of the natural enemies that have never been directly exposed to them (e.g., Tan et al. 2012; Pakyari and Enkegaard 2015; Abreu et al. 2014; Passos et al. 2018). As such, adult emergence and longevity were decreased when fourth-instars *B. cockerelli* parasitized with *T. triozae* eggs, larvae or pupae were either sprayed or dipped in insecticide solutions (Luna-Cruz et al. 2011; Martínez et al. 2015; Morales et al. 2018). Similarly, the percentage of females emerged was only affected when they came from exposed larvae to soybean oil and imidacloprid (Morales et al. 2018).

In Mexico, many efforts are being devoted to develop *T. triozae* as a commercial biological control agent of *B. cockerelli*. Therefore, there is an urgent need to ascertaining the effects caused by the most commonly applied insecticides to control this pest (e.g., soybean oil, abamectin, and imidacloprid), before the natural enemy can be included in pest management programs (Martínez et al. 2015; Morales et al. 2018). In this study, transgenerational effects on parasitism, host feeding, and sex ratio have been investigated in deep. A decrease in parasitism rate and a sex ratio biased towards males can seriously compromised the performance of *T. triozae* as biological control agent. In addition, a decrease on host feeding can also impaired reproduction if females are unable to get the nutrients they need for egg maturation.

Materials and Methods

Plant, psyllid, and parasitoid cultures

Tomato plants (Río Grande variety) were grown in a greenhouse (16-30 °C, 60% relative humidity [RH]), and natural light), irrigated as necessary, fertilized once a week with a nutritive solution ($Ca[NO_3]_2$, $MgSO_4$, KH_2PO_4, KNO_3, and K_2SO_4) (Steiner 1984) and micronutrients (Iron, manganese, boron, zinc, copper, and molybdenum) (Kelatex-Multi®, San Nicolás de Los Garza, Nuevo León, Mexico). The plants were allowed to grow for four weeks before being used in psyllid rearing.

Bactericera cockerelli and *T. triozae* were obtained, in 2014, from Instituto de Fitosanidad of the Colegio de Posgraduados in Montecillo (Estado de México, Mexico). Subsequently, the colonies were established in the Instituto de Investigaciones Agropecuarias y Forestales (IIAF), Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), El Trébol, Michoacán, Mexico. Nymphs and adults of *B. cockerelli* were reared on five potted tomato plants (Río Grande variety) inside three frame boxes (80 × 80 × 50 cm) covered by a mesh screen in the same greenhouse under the environmental conditions mentioned above. New host plants were supplied as needed.

The parasitoid *T. triozae* was reared in the laboratory conditions on tomato plants infested with a mixture of third-, fourth-, and fifth-instars of the host *B. cockerelli*, inside box frames similar to those described above (Morales et al. 2013, 2018; Martínez et al. 2015). Adults were provided with honey *ad libitum*. Under these conditions the parasitoid life cycle was completed in 11-13 days.

Insecticides

The commercial products AbaKrone® (1.8% abamectin, emulsion concentrate), ImidaKrone® (30.2% imidacloprid, suspension concentrate), and EPA 90® (90% refined soybean seed oil, emulsion concentrate) were used in this study. The three pesticides were obtained from Biokrone S. A. de C. V., Celaya, Guanajuato, Mexico. The solutions were prepared in distilled water with 0.01% Tween® 20 (nonionic tensoactive; polyoxyethylene (20) sorbitan monolaurate solution; Sigma Aldrich) in order to obtain a better solubilization of the tested insecticides as well as to enhance their adhesiveness.

Abamectin, with contact and translaminar action, primarily comprises two insecticidal neurotoxic macrocyclic lactones (B_{1a} and B_{1b}) produced by the soil actinomycete *Streptomyces avermitilis* (ex Burg et al.) Kim and Goodfellow (Ananiev et al. 2002; Kodandaram et al. 2014). This compound disrupts the postsynaptic GABA receptor and blocks signal transmission at the neuromuscular junction, causing paralysis and death in insects (Lasota and Dybas 1991). Imidacloprid is a neonicotinoid effective by contact and ingestion (Elbert et al. 2008) that binds to post-synaptic nicotinic acetylcholine receptors in insects disrupting synapses between neurons (Cloyd and Bethke 2010). The biorational insecticide, EPA, is a refined-oil extracted from soybean [*Glycine max* (L.)] seeds. Insecticide oils-based cause insect death by suffocation preventing the gaseous exchange by obstructing the spiracles (Martín-López et al. 2006). Also, oil products can impair the epidermal cell membrane function or structure (Bográn et al. 2014; Helmy et al. 2012).

Parasitism, host feeding, and sex ratio

To obtain adults of *T. triozae* for the bioassays, six tomato leaflets with ten fourth instar *B. cockerelli* parasitized with eggs (<10 h old), larvae (<12 h old) or pupae (<12 h old) of the parasitoid were dipped for 5 s into one of three concentrations of abamectin [9, 4.5, or 0.03 mg of active ingredient (a.i. /L)], imidacloprid (260, 130, or 3 mg a.i./L), or soybean oil (1.620, 810, or 443 mg a.i./L). The concentrations bioassayed correspond to the minimum field-registered concentration (MiFRC), ½MiFRC and the median lethal concentration (LC_{50}) for *B. cockerelli* fourth-instar nymphs, previously reported as the most preferred instar by *T. triozae* (Caudillo 2010). The MiFRC and ½MiFRC are the concentrations recommended by the manufacturer in Mexico to control *B. cockerelli*. The LC_{50} values were included because they had been previously estimated for fourth instars *B. cockerelli* (Bujanos et al. 2005). A similar number of tomato leaflets treated with distilled water plus surfactant Tween® 20 at 0.01% was used as control. After treatment, each tomato leaflet was individually transferred to an insect-free frame box (14 × 14 × 14 cm) and maintained under the laboratory conditions described above until the emergence of *T. triozae* adults (first generation, F1).

Based on our previous knowledge (Martínez et al. 2015), *T. triozae* females of the same age, mated and with experience of oviposition and host feeding were used for the experiments. To obtain them, immediately after emergence of the F1 generation, a group of 20 *T. triozae* adults (10 females and 10 males) emerged on the same day in each concentration, insecticide, and immature developmental treated stage (egg, larva or pupa) was placed in a cylindrical plastic tube (14 cm diameter × 35 cm height) with a tomato shoot with two-three leaves infested with a mixture of the different instars of the host *B. cockerelli* (first- to fifth-instars) during 13 days. To maintain turgor, the tomato shoot was placed in the nutritive solution of Steiner (1984) mentioned above in a plastic trough (4 cm diameter × 4 cm height) covered with Parafilm. The top and the bottom of the cylinder were covered with a fine mesh screen to permit air circulation and to prevent escape of the insects. Adults were fed on pure honey applied to the walls with a brush, and water was offered *ad libitum* in a plastic trough covered by Parafilm with a piece of wipe.

The bioassay consisted of six replicates of one *T. triozae* female 13-d-old, taken at random from each concentration, insecticide, and developmental stage treated. Each female was placed inside a cylindrical plastic tube, described above, with a tomato leaflet infested with a mixture of 24 second-, third-, fourth- and fifth-instar *B. cockerelli* nymphs (≤ 24-h-old after ecdysis) (six per instar) and provided with honey and water as described above. The petiole of the leaflet was maintained in a nutritive solution to prevent dehydration as mentioned above.

After 48 h exposure, the *B. cockerelli* nymphs were examined under a stereoscopic microscope and the number of parasitized nymphs and nymphs showing evidence of host feeding by *T. triozae* (nymphs appeared flat and desiccated because they were not filled with hemolymph and sometimes exhibited an inverted V shape) were recorded. The parasitoid development on *B. cockerelli* nymphs was followed until adult emergence (F2) and the adult sex ratio was determined.

Data analysis

Parasitism and host feeding were analyzed independently. In both cases, the experimental design consisted of a factorial of four treatments (control, abamectin, imidacloprid, and soybean oil), three concentrations (MiFRC, ½MiFRC, LC_{50}), three parasitoid developmental stages (egg, larva, and pupa) and four nymphal stages of the host (second-, third-, fourth-, and fifth-instar). The sex ratio was analyzed using a binomial distribution model. A generalized linear model procedure (PROC GLM), with the least significant difference (LSD) multiple range test ($p < 0.05$) to separate means, after checking for normality (PROC UNIVARIATE) and homoscedasticity (PROC GLM) of data in accord with Anderson-Darling and Bartlett, respectively, was used for analysis (Zar 2014). Data from females derived from eggs treated with 260 mg a.i./L of imidacloprid were not included in the analysis because all emerged adults were males. Data from females derived from eggs and larvae treated with 4.5 or 9 mg a.i./L of abamectin could not be analyzed because 100% of mortality was recorded in the parasitized host. All statistical tests were performed using SAS/STAT (version 9.4; SAS Institute, Cary, NC). All data are expressed as the mean ± SE.

Results

Parasitism

No parasitism of the host *B. cockerelli* second-instars was recorded when *T. triozae* females were treated as eggs, larvae, and pupae with any of the insecticides. However, in the other *B. cockerelli* instars, the parasitism by *T. triozae* was related to the concentration of the insecticides tested (Table 1). Concerning soybean oil and imidacloprid, parasitism of third instars of *B. cockerelli* did not significantly differ from that of the control, irrespective of the stage when parasitoid females were exposed (as egg or larvae). The only exception was recorded in the percentage of parasitism (16.7%) of females that emerged from eggs treated with the LC_{50} of imidacloprid (3 mg a.i./L), which was significantly different from the control (37.5%) ($F_{23,74} = 7.02$, $p < 0.001$) (Table 1). Regarding soybean oil and imidacloprid, parasitism of *B. cockerelli* third instars varied between 25.0 and 50.0% and between 22.2 and 44.4% and on fourth-instar of the host between 73.3 and 83.3% and between 83.3 and 96.3% when females were treated as eggs and larvae, respectively (Table 1). Regarding abamectin, parasitism by females from treated eggs and larvae could only be assessed at the LC_{50} (0.03 mg a.i./L) because 100% mortality of host nymphs was recorded at the other two concentrations evaluated (½MiFRC and MiFRC). When females were exposed to abamectin LC_{50}, the parasitism rate was significantly reduced compared to the control on third (5.5%; $F_{23,74} = 7.02$, $p = 0.022$) but not on fourth instars of the host (66.6%) when females were exposed as eggs (Table 1), and on third or fourth instars of the host (41.6 and 96.2%, respectively) when females were exposed as larvae (Table 1). In the control treatment, parasitism ranged between 37.5 and 30.5% on third instars and between 71.8 and 94.4% on fourth instars of the host when wasps were treated in the egg and larval stages, respectively (Table 1).

Table 1. Mean parasitism success (±SE) of *Tamarixia triozae* when exposed to three insecticides as egg, larva or pupa, on different nymphal instar (N) of *Bactericera cockerelli*.

Nymphal instar	**Treatment (concentration in mg a.i./L)**									
	Control	**Soybean oil**			**Imidacloprid**			**Abamectin**		
	0	**LC_{50}**	**½MiFRC**	**MiFRC**	**LC_{50}**	**½MiFRC**	**MiFRC**	**LC_{50}**	**½MiFRC**	**MiFRC**
Females derived from exposed eggs[a]										
N_3	37.5±12.4Acd	50.0±9.6Ad	44.4±11.1Acd	33.3±16.7Abcd	16.7±10.5Aab	41.7±5.7Acd	25.0±9.4Abc	5.5±5.5Aa	--	--
N_4	71.8±8.0Ba	79.1±12.4Ba	83.3±11.8Ba	83.3±0.0Ba	73.3±6.7Ba	74.1±9.4Ba	75.0±8.3Ba	66.6±0.0Ba	--	--
N_5	75.0±8.3Bc	77.8±5.5Bc	54.1±4.1Ab	33.3±5.3Ab	33.3±6.8Ab	38.9±8.2Ab	41.6±10.7Ab	0.0±0.0Aa	--	--
Females derived from exposed larvae[b]										
N_3	30.5±8.3Aa	44.4±11.1Aa	22.2±11.1Aa	37.0±3.7Aa	31.1±4.1Aa	33.3±6.0Aa	38.8±6.2Aa	41.6±2.7Aa	--	--
N_4	94.4±3.2Ca	88.8 ± 6.4Ba	80.5±15.9Ba	96.3±3.7Ba	90.7±6.0Ca	83.3±7.4Ba	85.1±6.2Ba	96.2±3.7Ba	--	--
N_5	72.2±9.6Bb	81.4 ±3.7Bb	74.1±9.7Ab	55.5±19.2Aab	49.9±11.0Ba	38.8±11.5Aa	33.3±12.0Aa	46.6±4.1Aa	--	--
Females derived from exposed pupae[c]										
N_3	26.6±7.5Ab	37.0±3.7ABbc	37.7±6.6Abc	33.3±6.4ABb	59.2±7.4Bc	17.7±4.4Aab	17.7±4.4Aab	11.1±0.0Aab	5.5±3.2Aa	11.1±6.4Aab
N_4	71.1±5.6Bb	57.7±5.4Bab	66.6±8.6Bb	42.5±9.6Ba	64.4±4.1Bab	55.5±12.8Bab	68.5±3.4Bb	41.6±5.3Ba	57.4±13.8Bab	58.3±13.8Bab
N_5	36.1±9.4Aab	35.1±7.2Aab	40.7±9.8Aab	18.5±3.7Aab	33.3±7.8Aab	44.4±12.8Bb	29.6±5.4Aab	11.1±0.0Aa	13.8±10.5Aa	16.6±13.2Aa

For females derived from each immature treated stage, means within columns (capital letters) and within rows (lower letters) followed by the same letter do not differ significantly ($p < 0.05$; GLM, LSD). $^{a}F_{23,74} = 7.02$, $p < 0.001$; $^{b}F_{23,78} = 9.23$, $p < 0.001$; $^{c}F_{29,102} = 4.97$, $p < 0.001$.

When *T. triozae* females were treated as eggs, the parasitism on the host *B. cockerelli* fifth instars was significantly reduced by every compound and concentration compared to the control ($F_{23,74} = 7.02$, $p < 0.001$), except the LC_{50} of the soybean oil (443 mg a.i./L) (77.8 *vs* 75.0% in the control) (Table 1). However, when treated in the larval stage, parasitism was significantly reduced ($F_{23,78} = 9.23$, $p < 0.001$) in all three concentrations of imidacloprid (33.3-49.9%) and abamectin (46.6%) compared to the control (72.2%) (Table 1).

In general, when *T. triozae* females were treated with the three pesticides as pupae, there was no significant influence on parasitism by female wasps on third-, fourth-, and fifth-instars of the host *B. cockerelli* except in two cases. Surprising, females treated with the LC_{50} of imidacloprid parasitized significantly more third instars (59.2%) and those treated with the ½MiFRC of abamectin parasitized significantly fewer third instars of the host (5.5%) than the control (26.6%) ($F_{29,102} = 4.97$, $p < 0.001$) (Table 1). Parasitism of fourth *B. cockerelli* instars, was significantly reduced by exposure to the MiFRC of soybean oil (42.5%) and the LC_{50} of abamectin (41.6%) compared to the control (71.1%) ($p < 0.001$) (Table 1).

Regardless of the treated parasitoid stage, parasitism among instars for all insecticides and control was in general significantly higher ($F_{2,329} = 114.60$, $p < 0.001$) on fourth instars of the host (41.6-96.2%), followed by fifth (0.0-81.4%) and third instars (5.5-59.2%) (Table 1).

Host feeding

Host feeding by *T. triozae* females on *B. cockerelli* was differently affected ($p < 0.01$) depending on the pesticide and concentration, the immature stage exposed to them and the nymphal developmental stage of the host where they fed (Table 2).

When *T. triozae* was exposed as eggs, feeding of females on second-instars *B. cockerelli* was significantly reduced by 46.6% in imidacloprid (½MiFRC, MiFRC) and by 33.3% in abamectin (LC_{50}) compared to controls (63.3%); feeding on third-instars was only significantly ($F_{31,101} = 12.86$, $p < 0.001$) reduced by the three concentrations of soybean oil (between 12.5 and 16.6%) and the LC_{50} of abamectin (16.6%) compared to the control (41.6%); feeding on fourth- or fifth-instars was not affected (Table 2).

Table 2. Host feeding of *Tamarixia triozae* females (number of *Bactericera cockerelli* nymphs consumed ± SE) when emerged from eggs, larvae or pupae exposed to the three insecticides on different nymphal instars (N) of *Bactericera cockerelli*.

	Treatment (concentration in mg a.i./L)									
Nymphal instar	**Control**	**Soybean oil**			**Imidacloprid**			**Abamectin**		
	0	**LC_{50}**	**½MiFRC**	**MiFRC**	**LC_{50}**	**½MiFRC**	**MiFRC**	**LC_{50}**	**½MiFRC**	**MiFRC**
Females derived from exposed eggs[a]										
N_2	63.3±11.0Cc	58.3±10.5Bbc	66.6±0.0Bbc	58.3±8.3Bbc	55.5±9.2Bbc	46.6±6.2Bab	46.6±9.7Bab	33.33±0.00Ba	--	--
N_3	41.6±8.3Bb	16.6±0.0Aa	16.6±9.6Aa	12.5±7.9Aa	50.0±6.8Bb	41.6±4.8Bb	33.3±10.5Bab	16.6±0.0ABa	--	--
N_4	3.3±3.3Aa	11.1±11.1Aa	0.0±0.0Aa	0.0±0.0Aa	10.0±4.0Aa	6.6±4.0Aa	4.1±4.1Aa	0.0±0.0Aa	--	--
N_5	0.0±0.0Aa	4.1±4.1Aa	4.1±4.1Aa	16.6±0.0Aa	5.5±3.5Aa	0.0±0.0Aa	2.7±2.7Aa	0.0±0.0Aa	--	--
Females derived from exposed larvae[b]										
N_2	57.7±11.3Bc	40.7±3.7Bb	11.1±0.0Aa	14.8±7.4Aa	28.8±5.6Bab	22.2±7.8Bab	33.3±10.5Bb	26.6±2.7Bab	--	--
N_3	44.4±9.2Bc	40.7±16.1Bc	13.3±6.4Aa	14.8±3.7Aa	31.4±3.4Bbc	30.5±8.3Bbc	19.9±4.1Bab	25.9±4.6Bab	--	--
N_4	11.1±0.0Aab	22.2±6.4Ab	2.2±2.2Aa	2.7±2.7Aa	1.8±1.8Aa	4.4±2.7Aa	5.5±2.4Aa	1.8±1.8Aa	--	--
N_5	11.1±0.0Aa	18.5±3.7Aa	8.3±5.3Aa	11.1±0.0Aa	9.2±1.8Aa	6.6±2.7Aa	7.4±3.7Aa	9.2±4.4Aa	--	--
Females derived from exposed pupae[c]										
N_2	70.3±6.8Cc	50.0±3.7Cab	50.0±7.9Cab	42.2±6.4Ca	62.2±15.1Cbc	47.2 ± 2.7Cab	55.5 ± 11.1Cab	48.8 ± 5.6Cab	48.8 ± 5.6Cab	50.0 ± 3.2Cab
N_3	46.6±8.8Bb	31.4±1.8Ba	26.6±2.7Ba	20.0±10.7Ba	22.2 ± 4.9Ba	22.2 ± 4.9 Ba	22.2 ± 10.1Ba	24.4 ± 4.1Ba	24.4 ±5.4Ba	25.9 ± 3.7Ba
N_4	13.3±2.2Aa	1.8±1.8Aa	5.5±2.4Aa	2.7±2.7Aa	3.7 ± 2.3Aa	1.8 ± 1.8Aa	6.6 ± 2.7Aa	8.3 ± 5.3Aa	3.7 ± 2.3Aa	5.5 ± 3.2Aa
N_5	13.8±2.7Aa	1.8±1.8Aa	4.4±2.7Aa	11.1±0.0Ba	11.1 ± 4.9ABa	5.5 ± 3.2Aa	11.1 ± 0.0ABa	14.8± 9.7Ba	5.5 ± 3.2Aa	11.1 ± 0.0Ba

For females derived from each immature treated stage, means within columns (capital letters) and within rows (lower letters) followed by the same letter do not differ significantly ($p < 0.05$; GLM, LSD). [a]$F_{31,101} = 12.86$, $p < 0.001$; [b]$F_{31,114} = 6.87$, $p < 0.001$; [c]$F_{39,151} = 13.24$, $p < 0.001$.

When *T. triozae* was exposed as larvae, feeding on second-instars *B. cockerelli* was significantly ($F_{31,114} = 6.87$, $p < 0.001$) reduced by any concentration of soybean oil (from 11.1 to 40.7%), imidacloprid (from 22.2 to 33.3%), and abamectin (LC_{50}; 26.6%) compared to controls (57.7%); on third-instars was only reduced by soybean oil (½MiFRC and MiFRC; 13.3 and 14.8%, respectively), imidacloprid (MiFRC; 19.9%), and abamectin (LC_{50}; 25.9%) (Table 2).

When *T. triozae* females were exposed as pupae, host feeding on second and third instars of *B. cockerelli* was significantly reduced ($F_{39,151} = 13.24$, $p < 0.001$) with soybean oil (42.2-50.0% and 20.0-31.4% for second- and third-instars, respectively), imidacloprid (47.2-55.5% and 22.2% for second- and third-instars, respectively), and abamectin (48.8-50.0% and 24.4-25.9% for second- and third-instars, respectively), compared to the controls (70.3 and 46.6% for second- and third-instars, respectively) (Table 2). Feeding on fourth- or fifth-instars was not affected.

In general, *T. triozae* females consumed significantly more second-instars *B. cockerelli* (11.1 to 70.3%), than third-instars (12.5 to 50%), fourth-instars (0 to 22.2%) or fifth-instars (0-16.6%) regardless of whether they were exposed to the insecticides as eggs, larvae or pupae ($F_{3,466} = 223.52$, $p < 0.101$) (Table 2).

Sex ratio

The percentage of *T. triozae* females of the F2 generation was not significantly different from that of the control when the F1 was exposed as eggs (61.1 ± 20.0-84.6 ± 5.3%; $F_{6,17} = 1.28$, $p = 0.32$), larvae (70.1 ± 10.7-84.3 ± 5.6%; $F_{7,27} = 0.44$, $p = 0.87$) or pupae (61.3 ± 15.0-88.6 ± 6.1%; $F_{9,31} = 1.47$, $p = 0.20$) on the host to soybean oil, imidacloprid, and abamectin. The percentage of female offspring exceeded 60% (61.1-88.6%) in every concentration of the three insecticides irrespective of the immature stage exposed to the pesticide except to LC_{50} abamectin (0.03 mg a.i./L) that only reached 50.0 ± 0.0%.

Discussion

Insecticides can adversely affect several biological parameters of natural enemies such as orientation, parasitism, host feeding, development, reproduction, and sex ratio, among others

(Stark and Banks 2003). In parasitoids, these parameters can be altered by direct contact with the pesticide but also when the developmental stage (e.g., adults) comes from immature stages that have previously been in contact with pesticides, and even sublethal concentrations can have an effect (e.g., Schneider et al. 2004; Biondi et al. 2015; Lira et al. 2015; Tena et al. 2016; Francesena et al. 2017). The results of this study, confirmed our previous findings (Morales et al. 2018) that three of the most commonly used pesticides in solanaceous crops in Mexico (soybean oil, imidacloprid, and abamectin) impaired different biological parameters in *T. triozae* females when immatures were exposed on fourth-instar nymphs of *B. cockerelli*. In the present study, this conclusion is extensive to exposure on other nymphal ages. The parasitism and sex ratio were differently affected depending on the insecticide and concentration applied, but also on the parasitoid immature stage on the host when the pesticide exposure and on the nymphal instar of the host *B. cockerelli* exposed. The reason why the insecticides studied here caused some long-term effects in *T. triozae* when exposed as immature stages, is not clear, but we hypothesize that they could have been absorbed and accumulated in high rates in the parasitoid body as active metabolites as reported by Schneider et al (2003), and could have affected some physiological processes in the apparently unaffected specimens. This highlights the importance of no using mortality as end point in the studies of non-target effects of pesticides.

Parasitoid susceptibility to pesticides can differ enormously depending on whether the free-living adults or the immature stages are exposed, being the former stage usually much more affected than the last ones (Jacas and Viñuela 1994; Schneider et al. 2004; Medina et al. 2008; Stanley and Preetha 2016). However, in ectoparasitoids, immature stages are also exposed to pesticides, and the difference can disappear. As such, Martínez et al. (2015) reported that adults of our parasitoid were very affected by imidacloprid and abamectin, which caused 100% mortality at 48 h after exposure in glass vials and in our study we found that especially abamectin at MiFRC and ½MiFRC when eggs or larvae were exposed on *B. cockerelli* nymphs of different ages, totally prevented the adult emergence.

The parasitism of *T. triozae* on fifth instars of the host *B. cockerelli* when females of the parasitoid were exposed to soybean oil in the egg stage was reduced by 1.3- and 2.2-fold at ½MiFRC and MiFRC, respectively. The MiFRC, however, only reduced parasitism on fourth-instars of this psyllid by 1.6-fold when females were exposed as pupae. Other oil-based compounds can cause similar effects. *Chenopodium ambrosioides* nr. *ambrosiodes* L. (Chenopodiaceae) essential oil extract reduced the parasitism of third- and fourth-instars by 1.9-fold in the same parasitoid-host system that we studied (Liu et al. 2012), but differently to our experiments, these authors assessed parasitism in females that were treated with essential oil by ingestion in the adult stage. In this case, this effect could be related to the repellency activity exhibited by some secondary metabolites presents in the horticultural mineral oils as shown in adults of some pests (Yang et al. 2010; Yang et al. 2013; Deletre et al. 2014). However, in contrast, many essential oils have been reported to be harmless for parasitoids (Werdin et al. 2013; Tena et al. 2016; Tofangsazi et al. 2018) and any subletal effect on different biological parameters have been cited (Werdin et al. 2013; Tena et al. 2016; Silva et al. 2018). For instance, a concentration of 16 mg a.i./L of a mineral oil (Biolid E®) did not affected parasitism of *Aphytis melinus* (DeBach) (Hymenoptera: Aphelinidae) in third-instars of the oleander scale, *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) when the parasitoid had been treated by spraying in the larval stage (Biondi et al. 2015). Similarly, a concentration of 40.2 mg a.i./L of borax plus citrus oil (Prev-Am®) did not affect parasitism of third instar larvae of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) by *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae) when females of the parasitoid had emerged from a direct spray treatment of pupae (Biondi et al. 2013).

In our assays, imidacloprid reduced parasitism on *B. cockerelli* fifth instars by *T. triozae* females exposed as eggs or larvae between 1.8-2.2-fold and 1.4-2.1-fold, respectively, whereas parasitism on third instar nymphs was reduced by 2.2-fold when females were exposed as eggs with the lowest concentration (3 mg a.i./L). Similar to our results, parasitism by a closely-related species, *Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae) on *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae) fourth instars, decreased by 1.4-fold when the parasitoid was directly treated as pupae with 4.8 mg a.i./L of another neonicotinoid, thiamethoxam (Lombardi de Carvalho 2008). Imidacloprid reduced parasitism of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) by *Eretmocerus mundus* (Mercet) (Hymenoptera, Aphelinidae) (Sohrabi et al. 2013) and *Encarsia inaron* (Walker) (Hemiptera: Aphelinidae) by between 1.2 and 1.7-fold (Sohrabi et al. 2012) when female parasitoids were exposed as larvae or pupae to 62.5, 500 or 1000 mg a.i./L of the compound.

The LC_{50} of abamectin resulted in a 6.7-fold reduction or totally eliminated parasitism of third or fifth instars of *B. cockerelli*, respectively, when females of *T. triozae* were treated as eggs. However, when larvae or pupae were the stages exposed to the pesticide, the adverse effect was lessened and parasitism was only partially reduced in fourth (1.7-fold) and fifth-instars (1.5-fold) of the host. When exposed as pupae, treatment with the ½MiFRC resulted in decreased parasitism on third instars of *B. cockerelli* by 4.8-fold. Similarly, parasitism of third instar larvae of *T. absoluta* by *B. nigricans* was reduced 2.3-fold when females were exposed as pupae to the highest recommended concentration of emamectin benzoate (25.5 mg a.i./L; a member of the abamectin family of insecticides), but not when they were exposed to 13.5 mg a.i./L of abamectin (Biondi et al. 2013). Also, both abamectin and emamectin benzoate reduced *E. mundus* parasitism of whitefly nymphs *B. tabaci* between 1.5- and 2.7-fold when pupae were sprayed with the MFRC of both pesticides at rates of 12.8 or 18 mg a.i./L, respectively (Fernández et al. 2010, 2015), which in the case of abamectin, was higher than that used in our assays (9 mg a.i./L). In addition, the commercials formulations were also different, which might have had an influence on the results, as shown by Adán et al. (1998) for the active ingredient azadirachtin**.** It is well known that orientation and foraging behavior of the parasitoids depend on nervous transmissions, which are targeted by neurotoxic insecticides through different modes of action (Desneux et al. 2004). Therefore, the effects on parasitism of *T. triozae* female when exposed to imidacloprid and abamectin as egg, larvae or pupae were probably due to alterations on the orientation and foraging behavior of this parasitoid, as suggested by Amini and Saber (2018) [*Lysiphlebus fabarum* (Marshall) (Hymenoptera: Braconidae) treated with imidacloprid].

Host feeding is intimately associated with parasitoid nutritional ecology, as it is the primary means of obtaining nutritional resources for most female parasitoids (Ueno 1999). However, this behavior also has been widely recognized as beneficial in the suppression of populations of insect pests due to the additional mortality inflicted on the host population (Yang et al. 2012; Liu et al. 2015; Cheng et al. 2017). As a consequence, if this behavior is affected by extrinsic factors, the potential of the natural enemy for pest control can be decreased. *Tamarixia triozae* females with no history of pesticide exposure can consumed 58% of fourth- and fifth-instar nymphs of the host *B. cockerelli* during their life span (Cerón-González et al. 2014), but show a preference for middle instar hosts [third- (28%) > fourth-

(19%) > fifth-instar *B. cockerelli* nymphs (16%); Morales et al. 2013]. Our findings are in agreement with these results because the prevalence of *T. triozae* host feeding was inversely proportional to the nymphal development stage. Similarly, the closely-related species *T. radiata* exhibits a similar feeding pattern and feeds more on third (30%) than on fourth or fifth instars (10%) of *D. citri* (Tang and Huang 1991). A lower energy demand for handling young instars compared to older ones that are more sclerotized, or the quality of the nutrients provided, might account for this (Morales et al. 2013).

There is no published information on *T. triozae* host feeding effects in females exposed to pesticides in the immature stages. In our study, all the studied pesticides did not cause a significant decrease in parasitoid host feeding on fourth- and fifth-instars of the host *B. cockerelli,* irrespective of the developmental stage during which parasitoids were exposed to pesticides (eggs-larvae-pupae). In contrast, host feeding on younger *B. cockerelli* nymphs (second or third-instars), was altered in general by any pesticide. The results obtained in our trials are indicative that pesticides can modify *T. triozae* feeding patterns on young instars of *B. cockerelli*. Among the reasons, pesticides could have impaired the host location [e.g., *Psyttalia concolor* ((Szèpl.); Jacas and Viñuela 1994)] or could have decreased the egg production and therefore the need of feeding on the host as reported in other synovigenic parasitoids (Desneux et al. 2007). The reductions in host feeding that we have observed in *T. triozae* could compromise the enemy's efficacy and survival if they are unable to obtain the essential nutrients for egg development. The host feeding behavior of *T. triozae* females on *B. cockerelli* nymphs was also decreased when they were exposed as adult by contact to residues of spinosad and betacyfluthrin (Luna-Cruz 2014)**.**

In *T. triozae* populations there are usually more females (86–88%) than males (Morales et al. 2013, Rojas et al. 2015) and in our study this pattern did not change in the F2 generation after pesticide exposure of eggs, larvae or pupae. Similarly, this biological parameter in the F1 generation of *T. radiata* (Beloti et al. 2015) and in the F2 generation in *Habrobracon hebetor* (Say) (Rafiee-Dastjerdi et al. 2012) and *Bracon brevicornis* Fabricius (Hymenoptera: Braconidae) (Sayed et al. 2014) was not modified when adults came from exposed eggs, larvae or pupae to emamectin benzoate or to the neonicotinoids thiamethoxam and imidacloprid. The parasitoid *A. melinus* when exposed as larvae to a mineral oil did not show any alteration on the sex ratio of F2 generation (Biondi et al. 2015) and *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae) when exposed as egg-larvae, pre-pupae, and pupae to imidacloprid or acetamiprid neither (Carvalho et al. 2010).

In contrast, several studies have reported transgenerational effects of some insecticides on the sex ratio of hymenopteran parasitoids when insects were exposed as immature stages. At this regard, Biondi et al. (2013) reported that in the F1 generation, the number of *B. nigricans* females decreased when insects were exposed as pupae to abamectin and emamectin benzoate. The number of females in the F2 generation of the endoparasitoids *Encarsia inaron* (Walker) (Hymenoptera: Aphelinidae) (Sohrabi et al. 2012) and *Trichogramma galloi* (Zucchi) (Hymenoptera: Trichogrammatidae) (Abreu et al. 2014) was also significantly lower following exposure as egg-larval, larval, pre-pupal or pupal stages to imidacloprid, thiamethoxam or a mixture of lambda-cyhalothrin + thiamethoxam. These changes in the sex ratio have been attributed to male sterility or mating behavior alterations (Biondi et al. 2013). Differential mortality of sexes in the immature treated stages could also have accounted for the differences observed in the sex ratio and this demands further study.

In conclusion, the results show that if the immature stages of *T. triozae* are exposed to pesticides, there are several long-term effects on the parasitoid, dependent on the insecticide and the concentration applied, on the immature stage exposed and on the nymphal instar of *B. cockerelli* offered for parasitism. The parasitism success of females emerged from exposed immature stages, was especially impaired by abamectin if eggs or larvae were exposed. The host feeding was mostly decreased by abamectin followed by imidacloprid, especially if eggs were exposed to them. No transgenerational effects in terms of sex ratio were observed for any insecticide. In order to know which the most compatible insecticide for solanaceous crop is with the parasitoid, future studies under realistic conditions are being planned.

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Compliance with ethical standards

Conflict of Interest: The authors declare that they have no conflicts of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Foliar persistence and residual activity of four insecticides of different mode of action on the predator *Engytatus varians* (Hemiptera: Miridae)

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HIGHLIGHTS

- Degradation and toxicity of four insecticides on *E. varians* were determined.
- The residues of the four insecticides were present up to 40 days.
- The half-life of imidacloprid was higher with respect to the other insecticides.
- Imidacloprid and dimethoate were harmful to *E. varians* adults, but not flufenoxuron and spinosad.

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ABSTRACT

A greenhouse study was conducted to investigate the degradation kinetics of spinosad, flufenoxuron, dimethoate and imidacloprid in tomato (*Solanum lycopersicum* L.) foliage and their residual toxicity on *Engytatus varians* (Distant) (Hemiptera: Miridae), a predator of the tomato psyllid *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae). Insecticides were sprayed at 100% and 50% of their maximum field-registered concentrations (MFRC). Starting 6 h after spraying, leaf samples were taken every 10 d for 40 d and analyzed while *E. varians* adults were exposed to treated leaves to evaluate residual toxicity. Immediately after application at 100% MFRC, the residue concentrations were 73.34 $\mu g\ g^{-1}$ spinosyn A and 59.2 $\mu g\ g^{-1}$ spinosyn D, 9.21 $\mu g\ g^{-1}$ flufenoxuron, 71.49 $\mu g\ g^{-1}$ dimethoate and 31.74 $\mu g\ g^{-1}$ imidacloprid. At 50% MFRC, initial residue concentrations were between 75% and 90% those at 100% MFRC. The estimated half-life (DT_{50}) of spinosyns A and D, flufenoxuron, and dimethoate was between 34 and 40 d, while that of imidacloprid was 112 d. Flufenoxuron caused no mortality, while mortality due to spinosad was less than 10%, and only during the first 10 d. Mortality caused by either imidacloprid or dimethoate was around 100% up to 10 d after application, then decreased to around 30% after 40 d. Dimethoate toxicity was approximately proportional to residue concentration, while for imidacloprid there was an apparent threshold around 15 $\mu g\ g^{-1}$. These results can be used to establish periods harmless for release of *E. varians* in the control of *B. cockerelli* on tomato crops under greenhouse conditions.

1. Introduction

During the past 20 years, several species of zoophytophagous mirids have gained ground in Europe as biological control agents against different insect pests. For example, they have been used

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successfully against the whitefly *Trialeurodes vaporariorum* Westwood (Lucas and Alomar, 2002), sweet potato whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) (Gabarra et al., 1995), western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Shipp and Wang, 2006), the aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae) (Fantinou et al., 2009), and the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Abbas et al., 2014), among others.

Engytatus varians (Distant) (Hemiptera: Miridae), which is widely distributed in North (Madden and Chamberlin, 1945; Martínez et al., 2014), Central (Maes, 1998), and South America (Schuh, 1995), is another promising candidate for biological control. It is a natural enemy of various pests on tomato crop (Silva et al., 2018; Pérez-Aguilar et al., 2019) and has shown potential effectiveness against the tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Brazil (Silva et al., 2016) and the tomato psyllid *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae) in Mexico (Pérez-Aguilar et al., 2019). When released at rates of 1 or 4 adults per plant in spring under greenhouse conditions, it suppressed between ~80% and 94% the populations of nymphs and adults of both *B. cockerelli* (Pérez-Aguilar et al., 2019). Another mirid, *Dicyphus hesperus* Knight (Hemiptera: Miridae), reduced *B. cockerelli* and *B. tabaci*, in the same proportions when applied at a rate of 1 predator per plant in fall-winter and summer (Calvo et al., 2016). However, biological control using predator mirids is still incipient in Mexico and, as far as we know, *D. hesperus* and *E. varians*, are not yet commercially available.

In Mexico, chemical insecticides remain the principal technique of crop protection, and there is an urgent needed to develop integrated pest management (IPM) practices (Messelink et al., 2014; Barzman et al., 2015; van Lenteren et al., 2018). In addition to their known impacts on human's health, environmental pollution, pest resistance, among others (Goulson, 2013; Wasi et al., 2013; Özkara et al., 2016; Ndakidemi et al., 2016; Banerjee et al., 2012; Bakırcı and Hışıl, 2012; Quijano et al., 2016), broad-spectrum chemical insecticides also affect predators used in biological control (Martinou et al., 2014; Fernandes et al., 2016; Wanumen et al., 2016a; Passos et al., 2017; Pérez-Aguilar et al., 2018), which are usually more susceptible than the pests (Desneux et al., 2007; Ndakidemi et al., 2016), thereby reducing their performance as biological controls agents (Cloyd and Bethke, 2010; Talebi et al., 2008).

One of the first steps to design a biological control strategy is to evaluate the toxicity of insecticides used against pests on their predators. This must be done under greenhouse and field conditions, and it is necessary to measure not only the toxicity immediately after application, but also the permanence of the insecticide and the evolution of its toxicity with time. Up to now, most studies have focused on the immediate lethality through different exposure methods: residual contact with treated glass vials or Petri dishes (Studebaker and Kring, 2003; Pérez-Aguilar et al., 2018), ingestion through contaminated prey or plant tissues (Wanumen et al., 2016b), topical spraying (Tillman et al., 2002), and immersion treatment (Nakahira et al., 2010). For example, in laboratory conditions, 1.5-h-old residues of imidacloprid, thiamethoxam, abamectin and chlorfenapyr caused 90% mortality on both third instar nymphs and adults of *E. varians*, while under greenhouse conditions, the mortality of third instar nymphs was between 35 and 87% after exposure to 2-h-old residues of thiamethoxam, abamectin, and chlorfenapyr, but dropped to 17–27% with 12-d-old residues (Pérez-Aguilar et al., 2018).

In this study, the degradation kinetics of spinosad, flufenoxuron, imidacloprid, and dimethoate after application on tomato plants (*Solanum lycopersicum* L.) under greenhouse conditions and the evolution of their residual toxicity on adults of the mirid *E. varians* were evaluated.

2. Materials and methods

2.1. Plant material

Tomato plants (saladette type, variety Río Grande), were grown individually in black plastic bags (20 cm diameter × 25 cm high) containing humus-rich soil and fertirrigated twice a week with 500 mL of a 50% nutritive solution of macronutrients ($Ca[NO_3]_2$, $MgSO_4$, KH_2PO_4, KNO_3, and K_2SO_4) (Steiner, 1984) with micronutrients (Iron, manganese, boron, zinc, copper, and molybdenum) (Kelatex-Multi®, San Nicolás de Los Garza, Nuevo León, Mexico). The plants were grown until they reached about 70 cm height and 11 or 12 fully expanded true leaves.

2.2. *Engytatus varians* rearing

Insects used in these tests came from a colony of *E. varians* maintained in the Instituto de Investigaciones Agropecuarias y Forestales (IIAF), Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) (Tarímbaro, Michoacán, Mexico), with no history of insecticide exposure. Nymphs and adults of this predator were reared on tomato plants (grown as mentioned above), in frame boxes (50 × 50 × 50 cm) entirely covered by a mesh screen. Eggs of the grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae), deposited on tomato leaves were supplied as food to the adults and nymphs of *E. varians* (Pineda et al., 2016). A 5% (w v^{-1}) solution of sugar (Chedraui®, Xalapa, Veracruz, Mexico) was also supplied to the predator in Eppendorf tubes (1.5 mL) (Axygen®, Union City, CA, USA) with a piece of cotton and replaced every 3 d to prevent fungal growth. The predator rearing was maintained under laboratory conditions of ~25 °C, 56% RH, and a photoperiod of 12:12 h (L:D).

2.3. Insecticide application

Four insecticides were tested in this study: spinosad, flufenoxuron, imidacloprid, and dimethoate, each one applied at the maximum field-registered concentration (100% MFRC) and at half of that concentration (50% MFRC). These insecticides, commonly used in solanaceous crop in Mexico, differ in their mode of action (see Table 1 for detailed information on active ingredients [a.i.]).

Ninety tomato plants (grown as described above) were used in this experiment, with 10 plants per treatment, including a control with no insecticide. Insecticides were sprayed with a hand-held sprayer until runoff. To enhance the wetting of the leaves, the surfactant Tween 20® was used at 0.1% (w v^{-1}). Control plants were sprayed with distilled water plus 0.1% surfactant. In order to avoid contamination between treatments, insecticide was applied outdoors and the plants were moved into a greenhouse 6 h later, after the spray had dried.

2.4. Environmental conditions

After treatment, plants were maintained in a passively ventilated plastic-covered greenhouse, without heating nor supplemental light. In previous experiments, global radiation and EAS-weighted UV radiation (McKinlay and Diffey, 1987) were measured outside and inside the greenhouse, using a Davis Vantage Pro weather station. It was found that the plastic cover was responsible for a 30% reduction in visible radiation, and a 75% reduction of UV radiation.

During the time of the experiment (25 March 2017 to 04 May 2017), only the daily maximum and minimum temperatures within

Table 1
Insecticides, manufacturers, active ingredients, formulations and concentrations, used for foliar persistence and residual activity experiments.

Trade name (supplier)	Active ingredient (%, Formulation)	[c]IRAC	Chemical Class	Mode of action	Concn. (mg a.i. L^{-1})	
					50%	100%
Entrust® (Dow AgroSciences)	Spinosad (44.20, [a]CS)	5	Spinosyns	Nicotinic acetylcholine receptor (nAChR) allosteric modulators	360	720
Cascade 100CD® (Basf)	Flufenoxuron (8.72, [b]EC)	15	Benzoylphenylureas	Inhibitors of chitin synthesis	60	120
Confidor® (Bayer)	Imidacloprid (30.20, CS)	4A	Neonicotinoid	Nicotinic acetylcholine receptor (nAChR) competitive modulators	700	1400
Dimetri 400® (Tridente)	Dimethoate (38, EC)	1B	Organophosphate	Acetylcholinesterase (AChE) inhibitors	800	1600

50% and 100%, correspond to the half of the maximum field recommended concentration (½MFRC) and the MFRC, respectively.
[a] CS = Concentrated suspension.
[b] EC = Emulsifiable concentrate.
[c] Insecticide Resistance Action Committee.

the greenhouse were recorded. Their values fluctuated little throughout the experiment: the maximum daily temperature remained between 35 °C and 40 °C, with a corresponding relative humidity between 20% and 35%, while the minimum temperature was around 15 °C, with a corresponding relative humidity between 90% and 95%. Apart from this experiment, the greenhouse was mostly empty, which explained the low relative humidity during the day.

2.5. Residue extraction

Leaf sampling was done 6 h after treatment (taken as 0 d) and then continued at 10, 20, 30, and 40 d after treatment. Each sample consisted of 10 leaflets picked from the middle section of plants from the same treatment. The collected leaflets were immediately transported to the laboratory and frozen at −20 °C until analysis.

Spinosad, flufenoxuron, imidacloprid, and dimethoate residue extraction was carried out using the methods of Sántis et al. (2012), Senguttuvan et al. (2014), Wang et al. (2013), and Belal and Gomaa (1979) and repeated three times. Frozen tomato leaflets (3 g) were mixed, chopped, and then extracted with 20 mL of a solution that consisted of acetonitrile + methanol + distilled water (5.3 + 3.3 + 1.3 by volume). Next, the 20 mL of extraction solution were recovered in 50 mL-Falcon tubes and 1.5 mL were filtered using polyvinylidene fluoride 0.2 μm-filters (Millipore, Billerica, MA, USA). From this sample, 1 mL was placed in 2 mL-Bond Eluent sample prep solutions tubes, QuEChERS Dispersive SPE 2 mL, fruits and vegetables with pigments, AOAC (Agilent Technologies, Santa Clara, CA, USA), and shaking for 5 min using a vortex. After that, the sample was centrifuged for 5 min to 4000 rpm. Finally, samples were placed in vials and frozen at −20 °C until analysis. The recovery efficiency measured using insecticide standards was >90%.

2.6. Residue determination

Insecticide residues were quantified by the liquid chromatography-tandem mass spectrometry technique (LC-MS/MS), which consisted of an ACQUITY UPLC and Xevo TQ-S from Waters (Mildford, MA, USA). The LC-MS/MS measurement was carried out as follows: 1 μL of standard solution or samples previously filtrated were injected onto a reverse phase LC column (ACQUITY UPLC BEH C18 1.7 μm, 2.1 mm × 50 mm). The pesticide was eluted from the column using a gradient of (A) water containing 4 mM of ammonium-formate and 0.1% of formic acid and (B) acetonitrile 100%. Linear gradient from 90% A: 10% B to 60% A: 40% B until 4.0 min, finally 100% A: 0% B by 1 min plus for equilibration. Flow rate of 0.4 mL min^{-1} was used. The total run time (including injection) was 6 min. The column temperature was kept at 40 °C, while the samples in the autosampler were kept at 10 °C. For detection, the Xevo TQ-S was used MRM mode. Because spinosad consists of a mixture of spinosyns A and D, both were determined separately. The MS/MS transition specific parameters that were used are provided as follows: Transitions for spinosyn A, precursor ion (m z^{-1}) 732.3, product ion (m z^{-1}) 98.2 and 142.2 with collision energies (V) of 35 and 35 respectively; cone voltage (V) of 5; Dwell (s) of 0.020; Acquisition time from 2.75 to 3.6 min. Transitions for spinosyn D, precursor ion (m z^{-1}) 746.5, product ion (m z^{-1}) 98.2 and 142.2 with collision energies (V) of 45 and 25 respectively; cone voltage (V) of 5; Dwell (s) of 0.020; Acquisition time from 3.0 to 3.75 min. Transitions for flufenoxuron, precursor ion (m z^{-1}) 489.2, product ion (m z^{-1}) 141.2, 158.1 and 401.1 with collision energies (V) of 40, 15 and 20, respectively; cone voltage (V) of 2; Dwell (s) of 0.020; acquisition time from 3.7 to 4 min. Transitions for imidacloprid, precursor ion (m z^{-1}) 256.2, product ion (m z^{-1}) 175.2 and 209.2 with collision energies (V) of 15 and 15, respectively; cone voltage (V) of 5; Dwell (s) of 0.020; acquisition time from 1.1 to 1.4 min. Transitions for dimethoate, precursor ion (m z^{-1}) 230.1, product ion (m z^{-1}) 125.1 and 199.1 with collision energies (V) of 20 and 5 respectively; cone voltage (V) of 30; Dwell (s) of 0.020; acquisition time from 1.2 to 1.5 min.

All pesticides were measured using a capillary voltage (kV) of 2.20, de-solvation temperature 500 °C; de-solvation gas flow (N_2) (L h^{-1}) of 800; cone gas flow (L h^{-1}) of 50 and nebulizer gas flow (Bar) of 7.0. Calculations were performed using the ratio of the peak areas of the quantifier transition of pesticides. Analytical standard of spinosad (spinosyn A and spinosyn D, 98%) was acquired from ChemService (West Chester, PA, USA) and flufenoxuron, imidacloprid, and dimethoate (all of them with purity of 99%) from Restek Corporation (Bellefonte, PA, USA). According to the calibration curve, the quantification procedure resulted in a correlation coefficient $R^2 = 0.99$. The insecticides residues are expressed on a leaf fresh weight basis (μg g^{-1}).

2.7. Bioassays of residual activity

The activity of spinosad, flufenoxuron, imidacloprid, and dimethoate residues was determined on *E. varians* adults, which were exposed to tomato leaves at the same times the residue samples were taken (0, 10, 20, 30, and 40 d after application). For each test, four *E. varians* adults (two males and two females, less than 5 d old), selected randomly from the laboratory colony, were introduced in a clip cage (transparent plastic cup, base diameter 4 cm) attached to the lower surface of a tomato leaf. Each clip cage was considered a replicate and five replicates per treatment were done. Adults of the predator were fed with *S. cerealella* eggs, presented on a double-sided tape (1 cm^2) (Truper®, Jilotepec, Estado de México, Mexico) placed on an inner wall of the clip cage. Adult mortality (distinguishing between males and females) was recorded daily for 3 d (72 h). An insect was considered dead if it did not move when touched with a thin bristle brush.

The insecticides were classified into the toxicity categories proposed by IOBC Working Group "Pesticides and beneficial organisms" for this kind of test: class 1: harmless (<25%), class 2: slightly harmful (26%–50%), class 3: moderately harmful (51%–75%), and class 4: harmful (>76%) (Hassan, 1994; Sterk et al., 1999).

2.8. *Experimental design and data analysis*

The treated plants were placed in the greenhouse under a completely randomized design, and each insecticide/concentration combination was considered a treatment, with 10 replicates.

For each concentration of spinosad, flufenoxuron, imidacloprid, and dimethoate, a simple first-order model (exponential decrease) was fitted to describe the degradation kinetics (Boesten et al., 2006). The model equation was $M = M_0 \exp^{(-kt)}$, where M was the remaining concentration at time t, M_0 the estimated initial concentration and k the relative degradation rate. The model fitting and estimation of confidence intervals for model parameters were performed by linear least square minimization, using the nls function of the R program (v. 2.3–1, Development Core Team, R Foundation for Statistical Computing, http://www.R-project.org). To obtain a more accurate estimation of the degradation half-life, the same model was applied by pooling the data from both concentrations for each insecticide, assuming that k was independent of application concentration: $M = M_{50\%\mathrm{MFRC}} \exp^{(-kt)}$ and $M = M_{100\%\ \mathrm{MFRC}} \exp^{(-kt)}$ for 50% and 100% of the MFRC, with parameters $M_{50\%\ \mathrm{MFRC}}$, $M_{100\%\mathrm{MFRC}}$, and k. The degradation half-life was calculated as $DT_{50} = \ln(2)\ k^{-1}$.

The statistical analysis of insect mortality was done by a two-way ANOVA, considering insecticide treatment as a factor and residue age as a second factor. The sex of the insect was initially evaluated as a third factor, but then dropped from the analysis because in general no significant differences between sexes were found (with a few exceptions detailed in the Results section). The least significant difference (LSD) multiple range test ($P < 0.05$) was used to separate means (SAS/STAT version 9.4; SAS Institute, Cary, NC). The relation between residues concentration on tomato leaf and the mortality on *E. varians* was measured by the Person's correlation coefficient.

3. Results

3.1. *Residue analysis*

The residues concentration decreased with time for all insecticides and concentrations insecticides (Fig. 1). Shortly after application of spinosad at 100% MFRC (720 mg a.i. L^{-1}), residual concentrations were 73.34 $\mu g\ g^{-1}$ spinosyn A and 59.12 $\mu g\ g^{-1}$ spinosyn D. At 50% MFRC, they were only slightly lower: 60 $\mu g\ g^{-1}$ and 45.88 $\mu g\ g^{-1}$, respectively. After 40 d, the residues had dropped to 21.42 $\mu g\ g^{-1}$ spinosyn A and 20.71 $\mu g\ g^{-1}$ spinosyn D at 100% MFRC, and to 15.05 $\mu g\ g^{-1}$ spinosyn A and 15.39 $\mu g\ g^{-1}$ spinosyn D at 50% MFRC. However, most of this decrease was observed between 30 and 40 d, since at 30 d the concentrations of spinosyn A were still 49.98 $\mu g\ g^{-1}$ at 100% MFRC and 37.70 at 50% MFRC and those of spinosyn D 37.59 $\mu g\ g^{-1}$ and 34.17 $\mu g\ g^{-1}$, respectively.

For dimethoate, the initial residue concentrations were 71.49 $\mu g\ g^{-1}$ at 100% MFRC (1600 mg a.i. L^{-1}) and 60.08 $\mu g\ g^{-1}$ at 50% MFRC. After 40 days, they had dropped to 22.46 $\mu g\ g^{-1}$ and 13.72 $\mu g\ g^{-1}$, respectively (Fig. 1). As for spinosad, most of this decrease was observed after 30 d, when the residual concentrations were still 49.98 $\mu g\ g^{-1}$ at 100% MFRC and 49.10 at 50% MFRC.

Flufenoxuron presented a more regular degradation: the initial residue concentrations were 9.21 $\mu g\ g^{-1}$ at 100% MFRC (120 mg a.i. L^{-1}) and 7.33 $\mu g\ g^{-1}$ at 50% MFRC, then decreased regularly during the experiment to reach 4.60 and 3.10 $\mu g\ g^{-1}$ for 100% and 50% MFRC, respectively, in the residue of 40 d old (Fig. 1).

Imidacloprid, in contrast, presented little degradation. The initial residue concentrations were 31.74 $\mu g\ g^{-1}$ at 100% MFRC (1400 mg a.i. L^{-1}) and 28.32 $\mu g\ g^{-1}$ at 50% MFRC (700 mg a.i. L^{-1}). After 40 d, more than two thirds of these concentrations remained, with 23.75 and 21.63 $\mu g\ g^{-1}$ concentrations for 100% and 50% MFRC, respectively (Fig. 1).

When fitting a first order degradation kinetics for each concentration of each insecticide separately, no significant differences were found in the k parameter between application concentrations. Therefore, only results from the global fit are presented: the estimated DT_{50} were similar for the spinosyns A and D, with 35 d (confidence interval: $27 < DT_{50} < 48$) and 34 d (confidence interval: $26 < DT_{50} < 46$), respectively, for flufenoxuron with 40 d (confidence interval: $31 < DT_{50} < 53$), and for dimethoate with 36 d (confidence interval: $27 < DT_{50} < 52$). In contrast, DT_{50} was three to four times longer for imidacloprid with 112 d (confidence interval: $86 < DT_{50} < 163$).

3.2. *Residual activity*

Flufenoxuron did not cause mortality on *E. varians* adults in any of the treatments applied. Although spinosad around 10% mortality up to 10 d after application (but not statistically different from the control), this effect disappeared with older residues (Table 2).

In contrast, both imidacloprid and dimethoate caused between 80% and 100% mortality up to 20 d after application, in both application rate (with the exception the 20-d-old residue at the lowest concentration of dimethoate in which the mortality was 70%) (Table 2). The residual activity of imidacloprid and dimethoate subsequently decreased, with mortalities between 30% and 65% with 30- and 40-d-old residues under both application concentrations (Table 2). When taking into account the sex factor, it was found that imidacloprid and dimethoate had slightly different effects on males and females. A significantly higher mortality in males (between 50% and 80%) than in females (between 10% and 50%) was observed at 30 and 40 d after application of either compound at 50% MFRC, and 30 d after application of imidacloprid at 100% MFRC ($F = 24.64$; $df = 89, 288$; $P = 0.001$).

According to mortality criteria established by the IOBC, flufenoxuron and spinosad residues were classified as harmless (class 1) for all conditions assayed. In contrast, 0-, 10-, and 20-d-old residues of imidacloprid and dimethoate fell within the highest toxicity class (class 4, harmful), with the exception of the 20-d-old residue of 800 mg a.i. L^{-1} of dimethoate (class 3, moderately harmful) (Table 2). The 30- and 40-d-old residues of both compounds were classified as moderately harmful (class 3) and slightly harmful (class 2), respectively, with exception to the 30-d-old residue of 700 mg a.i. L^{-1} imidacloprid, which fell in class 2.

Finally, the correlations between residue concentration and mortality of *E. varians*, obtained after pooling all sampled concentrations and dates, showed that, independently from the applied concentration and residue age, mortality was strongly correlated with residue concentration. Excepted for flufenoxuron, which did not kill the insects, the correlations were significantly positive for spinosad ($r = 0.63$; $P = 0.046$) (Fig. 2A), imidacloprid ($r = 0.864$; $P = 0.001$) (Fig. 2B), and dimethoate ($r = 0.923$; $P = 0.001$) (Fig. 2C). Extrapolating the regression line suggested that, while for dimethoate mortality was roughly proportional to residue concentration, for imidacloprid and spinosad, no mortality would occur below a threshold around 15 $\mu g\ g^{-1}$ and 40 $\mu g\ g^{-1}$, respectively.

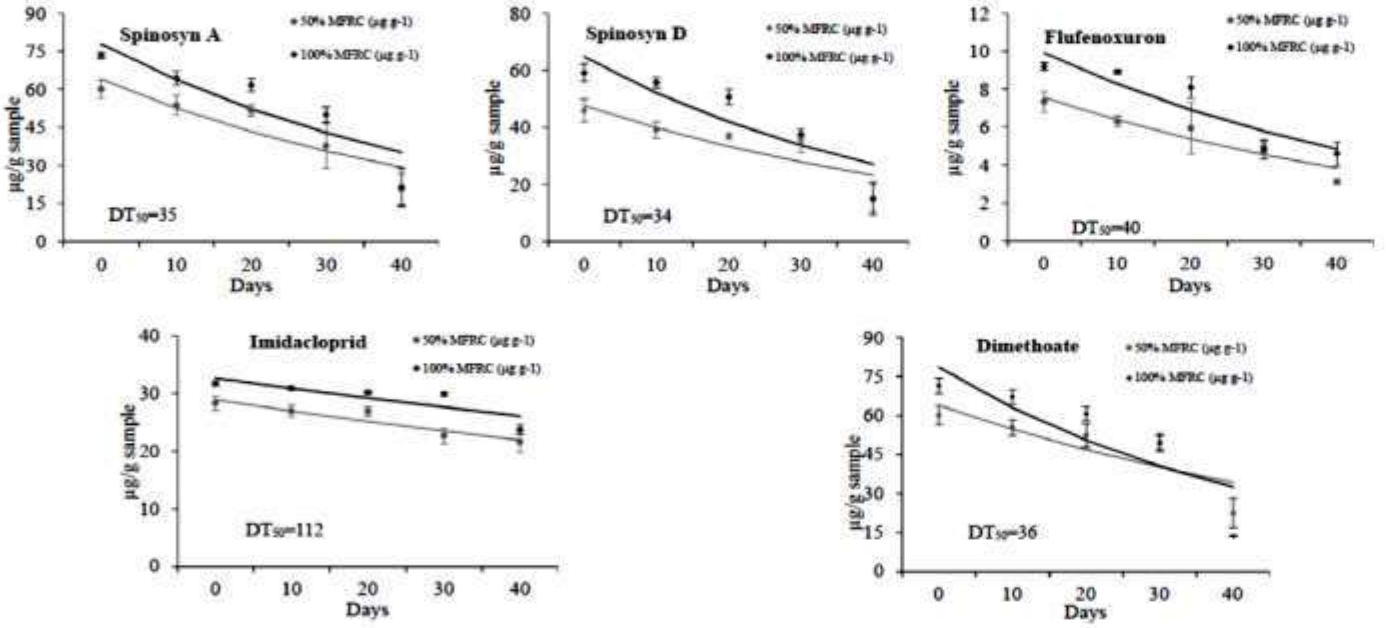

Fig. 1. Decline of insecticide residues over time following application. The black and grey color correspond to 100 and 50% MFRC applied, respectively. Data points represent the average residue concentration at different sampling times with corresponding standard error. Both lines represent the degradation kinetics of the corresponding treatment, fitted by a simple first-order (SFO) model.

Table 2
Mortality of *Engytatus varians* adults after 72 h of exposure to residues of different ages plants treated with spinosad, imidacloprid and dimethoate and toxicity categories according to Working Group "Pesticides and beneficial organisms of IOBC".

Treatment	Concentration (mg a.i. L^{-1})	Adult mortality (%)/Age of residue on leaves (IOBC Toxicity Class)				
		0 d	10 d	20 d	30 d	40 d
Control	0	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a
Spinosad	360	5 ± 5a (1)	0 ± 0a (1)	0 ± 0a (1)	0 ± 0a (1)	0 ± 0a (1)
	720	10 ± 6a (1)	10 ± 6a (1)	0 ± 0a (1)	0 ± 0a (1)	0 ± 0a (1)
Imidacloprid	700	100 ± 0b (4)	90 ± 6b (4)	80 ± 14bc (4)	35 ± 16b (2)	30 ± 9b (2)
	1400	100 ± 0b (4)	100 ± 0b (4)	85 ± 9bc (4)	65 ± 15c (3)	50 ± 11b (2)
Dimethoate	800	100 ± 0b (4)	100 ± 0b (4)	70 ± 14b (3)	55 ± 17bc (3)	30 ± 13b (2)
	1600	100 ± 0b (4)	100 ± 0b (4)	100 ± 0c (4)	65 ± 16c (3)	30 ± 19b (2)

Means within of each columns followed by the same letter do not differ significantly ($P < 0.05$; MIXED, LSD).

4. Discussion

In this study, we found that under greenhouse conditions, the concentrations of spinosyns A and D, flufenoxuron, and dimethoate residues on tomato leaves decreased with an estimated DT_{50} between 34 and 40 d and those of imidacloprid with a DT_{50} of 120 d. These are much slower degradation rates than found in other studies. Excepted for Sántis et al. (2012) who, under conditions similar to those of the present experiments, found a DT_{50} of 124 d for spinosad on pepper (*Capsicum annuum* L., Solanaceae]) leaves treated with both 60 and 120 mg a.i. L^{-1}, other authors found values of only a few days for all insecticides. For instance, when spinosad was applied on cabbage (*Brassica oleracea* var. *capitata* L., Brassicaceae) leaves at concentrations of 2.1, 4.4, and 42.6 mg a.i. L^{-1} the DT_{50} values were 3.7, 6.3, and 3.4 d, respectively (Sharma et al., 2008; Akbar et al., 2010).

For compounds within the same family of chitin synthesis inhibitors as flufenoxuron, the DT_{50} of diflubenzuron, triflumuron, hexaflumuron, flufenoxuron or lufenuron was ≤2.5 d on pepper fruits or cabbage leaves when applied at concentrations between 75 and 3240 mg a.i. L^{-1} under either greenhouse or field conditions (López-López et al., 2004; Senguttuvan et al., 2014). Concerning dimethoate, the DT_{50} was 2.5 d on cucumber (*Cucumis sativus* L., Cucurbitaceae]) leaves under greenhouse conditions (Geng et al., 2018) and 3.9 d on *B. oleracea* leaves under field conditions (Antonious et al., 2007) when applied at 1160 and 2783 mg a.i. L^{-1}, respectively. For imidacloprid, which presented the longer DT_{50} (120 d) in the present study, the discrepancy with other reports was even higher, with values up to 30 times longer. On lettuce (*Lactuca sativa* L., Asteraceae) (Itoiz et al., 2012) and cabbage (Khay et al., 2008) leaves, under greenhouse conditions, the DT_{50} was 4.4 and 3.2 d when applied at 138 and 50 mg a.i. L^{-1}, respectively. Under field conditions the DT_{50} of imidacloprid and thiamethoxam, another neonicotinoid, was between 2.5 and 4.4 d when applied on cabbage (Mukherjee and Gopal, 2000), tomato (Romeh et al., 2009), and tobacco foliage (Wang et al., 2013).

It is well known that the foliar persistence of chemical insecticides depends on the climatic conditions, type of application, plant species, dosage, the interval between application, and leaf age (Khay et al., 2008; Sántis et al., 2012; Jacobsen et al., 2015; López-López et al., 2004). However, the magnitude of the discrepancy between the degradation rates found in this and other studies is difficult to explain. Temperature was probably not a critical factor, since the rather high maximum temperature (~35–40 °C) in the present experiment should increase the rate of chemical reactions instead of slowing them. Relative humidity, which was low

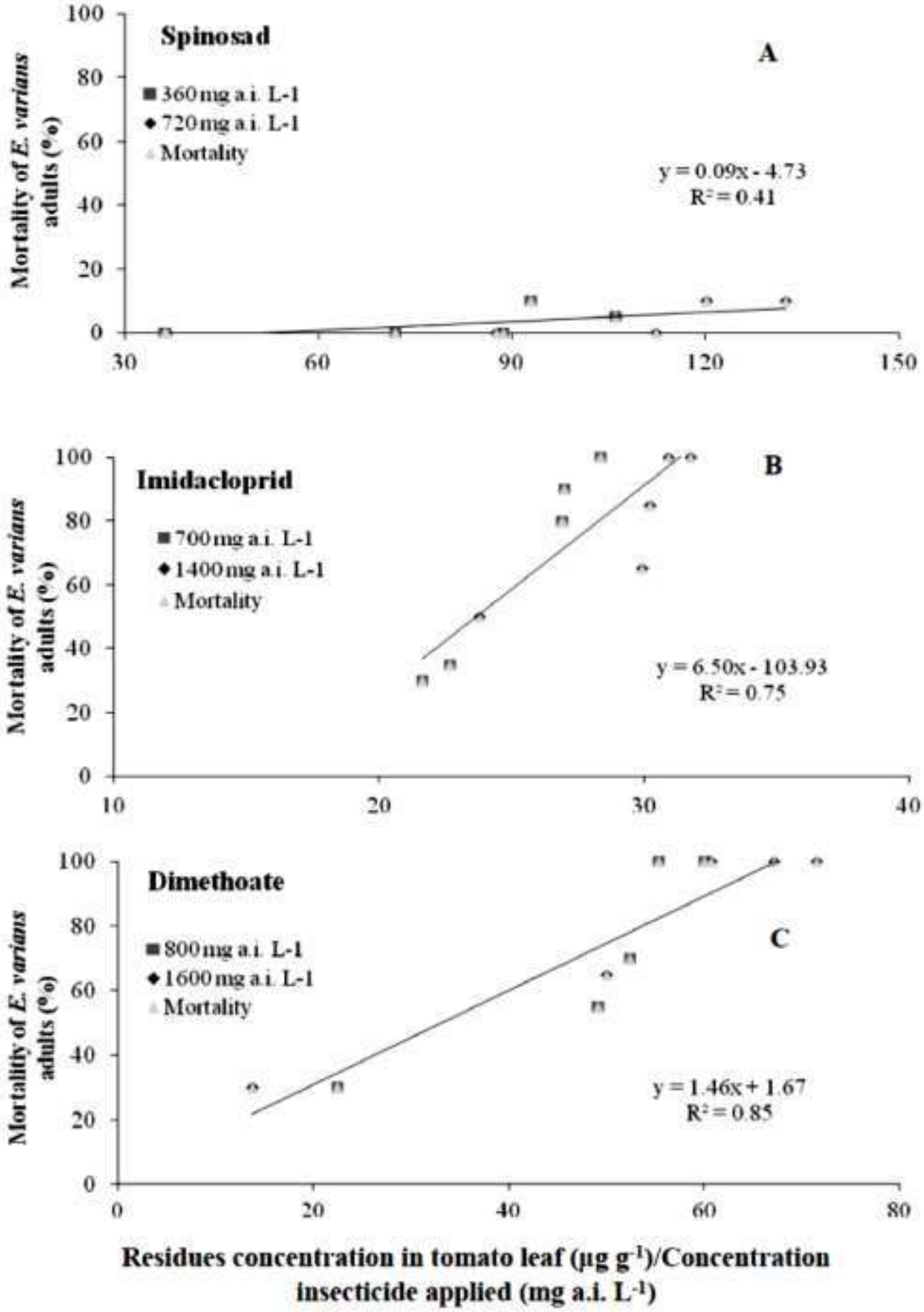

Fig. 2. Correlation between residues concentration of spinosad (A), imidacloprid (B), and dimethoate (C) in leaf and mortality of *Engytatus varians* adults.

throughout the experiment, may partly explain the low degradation rate of the chemical reactions involved happened in solution. However, Itoiz et al. (2012) and Khay et al. (2008) found high degradation rates of imidacloprid in similar conditions of temperature and relative humidity (16–35 °C and 70% in their studies against 15–40 °C and 20–90% in the present one). The most likely reason to explain the low degradation rate is the use of UV-filtering plastic, which reduces UV radiation by 75%, in our greenhouse. None of the greenhouse studies cited mentioned the intensity of UV radiation during the experiments, but it may have been higher than under the present conditions if they did not use UV filtering plastic (which is commonly sold on the tropical mountain climate of central Mexico, but not necessarily so under other climates). In the field, the UV intensity is obviously higher due to direct exposure to sunlight and causes a much faster degradation (Sántis et al., 2012).

Concerning toxicity, *E. varians* was very susceptible to both imidacloprid and dimethoate (IOBC classes 3 or 4, moderately harmful or harmful) even 30 d post-application (excepted for imidacloprid applied at 50% MFRC, which fell in the class 2, slightly harmful). For both insecticides, mortality appeared to be linearly related to residue concentration. For dimethoate, it increased almost proportionally to a concentration until 100% mortality was reached around 60 $\mu g\ g^{-1}$. For imidacloprid, extrapolating the trend line suggested that concentrations below 15 $\mu g\ g^{-1}$ would cause no mortality, while 100% mortality was reached around 30 μg g^{-1}.

Other authors have also found a high toxicity of imidacloprid or other neonicotinoids shortly after application, followed by a slow decrease during the following days. 100% mortality was found when *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) adults were exposed to *Chrysanthemum* L. (Asteraceae) foliage containing 1-h-old imidacloprid greenhouse-aged residue (Van De Veire et al., 2002) or when *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) adults were exposed to tomato foliage containing 7-, 14-, and 21-d-old field-aged thiamethoxam residue (Fernandes et al., 2016). Wanumen et al. (2016a) reported that the mortality of *Macrolophus basicornis* Stal (Hemiptera: Miridae) adults exposed to imidacloprid residues decreased linearly with residue age, from 100% at 2 h after applications down to 7% after 31 days.

Concerning dimethoate, there is no literature about its effects on mirid adults. However, other organophosphates presented high toxicity and slow degradation, both in the greenhouse and in the open field. In field tests, 21-d-old residues of chlorpyriphos and acephate, applied at 3.45 and 1406.25 mg a.i. L^{-1}, on tomato plants still caused 100% of *O. insidiosus* adults (Fernandes et al., 2016). In the greenhouse, exposure to 1-d-old residues of methamidophos caused more than 90% mortality on third-fourth instar nymphs of two mirid species, *Dicyphus tamaninii* Wagner and *Macrolophus caliginosus* (Wagner) (Hemiptera: Miridae) (Figuls et al., 1999). The mortality rate then decreased with the age of the residues to reach a minimum (16% for *D. tamaninii* and 36% for *M. caliginosus*) between 21 and 31 d after application.

In contrast to imidacloprid and dimethoate, spinosad and flufenoxuron proved to be safe for *E. varians* adults (IOBC class 1, harmless). Flufenoxuron had no effect on *E. varians* adults. Studies with other inhibitors of chitin synthesis had shown similar results on other mirid species: either in the greenhouse and the laboratory, 2-h-old residues of lufenuron, buprofezin, and teflubenzuron present in *Chrysanthemum*, tomato or kalanchoe (*Kalanchoe blossfeldiana* Poelln, Crassulaceae) leaves, caused a low mortality (≤8%) in *O. laevigatus* adults (Van De Veire et al., 2002), third-fourth instar nymphs of *D. tamaninii* (Castañe et al., 1996) or *Pilophorus typicus* (Distant) (Hemiptera: Miridae) adults (Nakahira et al., 2010). However, in other studies, a significant mortality (between 20 and 24%) had been observed on adults of *E. varians* (Pérez-Aguilar et al., 2018) and *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae) (Amarasekare and Shearer, 2013) exposed to 1.5-h- and 10-d-old residues of teflubenzuron and novaluron, another inhibitor of chitin synthesis. This higher toxicity may be explained by the fact that the insecticides were presented on glass substrate, where they are often more toxic than when applied on plants, as the cuticular waxes of plant leaves absorb insecticides (Aguirre et al., 2013), and reduce their availability (Desneux et al., 2005).

Concerning spinosad, residues less than 10-d-old caused less than 10% mortality, while older residues had no effect. Our findings agree with those of Arnó and Gabarra (2011) who found that under greenhouse conditions 2-h-old foliar residue of 120 mg a.i. L^{-1} of this compound caused less than 10% mortality on adults of the closely-related species *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae). Other studies, however, had found higher toxicity for spinosad: at higher application rates, when exposed to 1-h-old foliar residues of spinosad applied on greenhouse tomato sprouts at 1200 mg a.i. L^{-1} (50% higher than used in the present study), the mortality in *O. laevigatus* adults exposed was more than 75% and then decreased to 45% after 7 d and 25% after 14 d (Biondi et al., 2012). This decrease was attributed to a dilution in expanding leaves and to the high enzymatic activity present in young sprouts (Fantke and Juraske, 2013; Sun et al., 2015; López-López et al., 2004; Jacobsen et al., 2015).

5. Conclusions

In conclusion, this is the first report on compatibility of spinosad, flufenoxuron, imidacloprid, and dimethoate with *E. varians*. According to our results, imidacloprid and dimethoate should not be used in combination with the zoophytophagous predator *E. varians* in an IPM scheme, due to their long persistence and the high mortality they cause on adults (up to 65% of mortality, even 30 days after application). In contrast, spinosad and flufenoxuron were evaluated as safe and their use appears compatible with biological control of *E. varians*, at least on tomato crops in greenhouse conditions where it has showed high effectiveness as biological agent against *B. cockerelli* (Pérez-Aguilar et al., 2019). However, sublethal effects, which were not examined in the present study, should be expected from inhibitors of chitin synthesis and spinosad. For example, teflubenzuron decreased offspring and longevity in *E. varians* when exposed to 1-h-old residues (Pérez-Aguilar et al., 2018), and spinosad affected the offspring of *O. laevigatus* adults exposed to older residues (1-h-, 7-d-, and 14-d-old) (Biondi et al., 2012). Therefore, more studies should be carried out to verify that sublethal effects of spinosad and flufenoxuron would not compromise the performance of *E. varians* as biological control agents.

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Eco-toxicological risk of some insecticides on *Engytatus varians* (Hemiptera: Miridae), an important predator of *Bactericera cockerelli* (Hemiptera: Triozidae)

Abstract

Engytatus varians (Distant) (Hemiptera: Miridae) is a potential predator of the tomato psyllid, *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae), one of the most detrimental pests of several solanaceous crops. The control of *B. cockerelli* is done through use of chemical insecticides; therefore, it is necessary to know the effects caused by these compounds on *E. varians*. In this study, the mortality of *E. varians* adults, exposed on tomato, *Solanum lycopersicum* L., leaflets treated with three concentrations (minimum field-registered concentration [MiFRC], one-half the MiFRC [½MiFRC], and the median lethal concentration [LC_{50}] for *B. cockerelli* fourth-instars) of the insecticides abamectin, imidacloprid, bifentrhin, and flufenoxuron was determined. The ½MiFRC and MiFRC of bifentrhin, abamectin, and imidacloprid caused between 32-82, 30-98, and 44-100% of mortality on *E. varians* adults at 24, 48, and 72 h post-treatment, respectively, while in the LC_{50} of these three compounds the mortalty was observed between 2-18%. In contrast, flufenoxuron did no cause any effect on the adults of the predator. On the other hand, several biological parameters (longevity, predation, fecundity, and nymphal development) of the surviving *E. varians* females to the four insecticides mentioned above were also evaluated. Only the MiFRC of bifentrhin and the LC_{50} of abamectin, significantly decreased the longevity of females of this predator. *Engytatus varians* females exposed to the three concentrations of the four insecticides consumed significantly less *B. cockerelli* eggs, while than when a mixture of second- and third-instar (N_2-N_3) of this pest was offered, predation was only affected by the MiFRC of bifentrhin and LC_{50} of abamectin. Abamectin, bifentrhin, and flufenoxuron decreased the fecundity of the *E. varians* females and only the LC_{50} and ½MiFRC of this last insecticide delayed significantly the nymphal development. In conclusion, imidacloprid was harmful, while than abamectin and bifenthrin should be used with caution in areas where *E. varians* is present naturally or in areas where this predator could be released. Moreover, more studies should be conducted to evaluate the compatibility of flufenoxuron with this predator under more real conditions.

Key words: zoophytophagous predador, tomato psyllid, sublethal effects, biological parameters.

Morales S.I. et al. Eco-toxicological risk of some insecticides on *Engytatus varians* (Hemiptera: Miridae), an important predator of *Bactericera cockerelli* (Hemiptera: Triozidae). **En preparación para su publicación.**

Introduction

The use of several zoophytophagous mirid bugs (Heteroptera: Miridae) species as biological control agents of arthropod pests in protected crops has increased exponentially in the last 20 years, especially in Europe (Biondi et al. 2016, Gigon et al. 2016, Ingegno et al. 2017, van Lenteren et al. 2018). *Dicyphus tamaninii* Wagner (Lucas and Alomar 2002), *Dicyphus maroccanus* Wagner (Abbas et al. 2014), *Macrolophus pygmaeus* (Rambur) (Maselou et al. 2014), and *Nesidiocoris tenuis* (Uhler) (Calvo et al. 2012) are routinely used for controlling several agricultural pests such as the whiteflies *Trialeurodes vaporariorum* Westwood (Lucas and Alomar 2002) and *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) (Barge et al. 1995), western flower trips *Frankliniella occidentalis* (Pergande) (Shipp and Wang 2006), the aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae) (Fantinou et al. 2009), and the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Abbas et al. 2014).

In contrast, only three species of predatory mirids are recorded in Mexico: *Engytatus varians* (Distant) (Martínez et al. 2014), *Dicyphus hesperus*, Athias-Henriot (Calvo et al., 2016), and *N. tenuis* (Pineda et al. 2017). The releases under greenhouse conditions of *E. varians* and *D. hesperus* showed their potential as biological control agents on the whitefly *B. tabaci* or the tomato psyllid *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae) (Calvo et al. 2016, 2018; Pérez-Aguilar et al. 2019), both homopteran species considered as two of most important pests of several Solanaceae crops. In Mexico, *E. varians* was reported for the first time in 2014 feeding on *B. cockerelli* nymphs on tomato (*Solanum lycopersicum* L.) plants under greenhouse conditions (Martínez et al. 2014). Since then, several studies have been conducted on this predator (e.g., its biology; Pineda et al. 2016, population parameters and development on artificial diets; Palma-Castillo et al. 2019, predation under laboratory and greenhouse conditions; Velázquez-Rodríguez et al. 2015, Pérez-Aguilar et al. 2019, and damages caused to tomato fruits, Esquivel-Ayala 2018).

Under laboratory conditions, it was demonstrated that fourth instar (N_4) and adults of *E. varians* preyed on 46 and 75% of the third instar (N_3) of *B. cockerelli*, respectively (Martínez et al. 2014, Velázquez-Rodríguez et al. 2015). In addition, under greenhouse conditions, the releases of 1 and 4 adults of *E. varians* per tomato plant reduced 80 and 90%

the populations of nymphs and adults of *B. cockerelli*, respectively (Pérez-Aguilar et al. 2019). On the other hand, chemical control is the most widely used agricultural practice for the control of *B. cockerelli*. Some of the most commonly used toxicological groups of insecticides to reduce the populations of this pest are avermectins, neonicotinoids, pyrethroids, organophosphorus, spinosyns, and insect growth regulators (Cerna et al. 2013; Dávila-Medina et al. 2012; Garzón-Begrimed et al. 2007; Vega-Gutiérrez et al. 2008).

Little information is available about the effects that chemical insecticides may cause on the natural enemies of *B. cockerelli*. *Engytatus varians* is a potential biological control agent for this pest; therefor, before proposing its use, in combination with chemical insecticides, it is necessary to know the lethal (mortality) and sublethal effects (development, adult longevity, fecundity, and sex ratio, among others) (Desneux et al. 2007; Stark et al. 2007) that these compounds can to cause on this predator. Recently it was demonstrated that the insecticides chlorfenapyr, thiamethoxam, and abamectin caused ~ 90% of mortality on third instar and adults of *E. varians*. In contrast, teflubenzuron was the insecticide that caused the lowest mortality on both development stages (24 and 66%, respectively) (Pérez-Aguilar et al. 2018). However, mortality should not be the only parameter to be considered as endpoint when effects of chemical insecticides on natural enemies are evaluated (Schneider et al. 2008). Therefore, the effects of chemical insecticides in the long term should be studied because they could compromise the performance of any biological control agent.

In this study, the lethal (mortality) and sublethal effects (longevity, predation, fecundity, and nymphal development) caused by four insecticides (abamectin, imidacloprid, bifenthrin, and flufenoxuron), commonly used in tomato production in Mexico, on the adults of *E. varians* were determined.

Materials and Methods

Insects and rearing

Insects used in these tests came from colonies of *B. cockerelli* and *E. varians* maintained in the Entomology Laboratory of the Instituto de Investigaciones Agropecuarias y Forestales (IIAF) of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) (El Trébol, Michoacán, Mexico). These insect colonies had no history of insecticide exposure. Nymphs and adults of *B. cockerelli* were reared on tomato plants (variety Río Grande) (~30 cm in height with 11-15 fully expanded leaves) in three frame boxes (50 × 50 × 50 cm) entirely covered by a mesh screen. New host plants were supplied as needed. The entire rearing process of the tomato psyllid was completed at ~25 °C and 56% RH, with a photoperiod of ~12:12 h (L:D).

Nymphs and adults of *E. varians* were maintained in three frame boxes, identical to those described above, on tomato plants. Every four days adults and nymphs were provided with eggs of the grain moth *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) (Bio Bich de Mexico, S.A. de C.V., Uruapan, Michoacán, Mexico), deposited on tomato leaves, and supplemented with a 5% (w/v) solution of sugar (Chedraui®, Xalapa, Veracruz, Mexico) in Eppendorf tubes (1.5 ml) (Axygen®) with a piece of cotton. Predator rearing was maintained under the same conditions as those of its prey *B. cockerelli.*

Insecticides

The commercial products Agrimec® (1.8% abamectin [an allosteric modulator of the Cl channels, in the postsynaptic neuron receptors], emulsion concentrate, Agroquímicos de Michoacán S.A. de C.V., Morelia, Michoacán, Mexico), Confidor® (30.2% imidacloprid [a competitive modulator of the nicotinic acetylcholine receptor], suspension concentrate, Bayer de México S.A. de C.V., Ciudad de México, Mexico), Talstar® 100 (12.5% bifenthrin [a modulator of the sodium channel, in the axon of the neuron], emulsion concentrate, FMC Agroquímica de México, S. de R. L. de C.V., Zapopan, Jalisco, Mexico), and Cascade® 100CD (8.72% flufenoxuron [a chitin biosynthesis inhibitor], emulsion concentrate, Basf Mexicana S.A. de C.V., Ciudad de México, Mexico) were used in this study.

Adult mortality

Sixty-five tomato leaflets were dipped for 5 s into one of three concentrations of abamectin [9, 4.5, or 0.03 mg of active ingredient (a.i./L)], imidacloprid (260, 130, or 3 mg a.i./L), bifenthrin (240, 120 or 8 mg a.i./L) or flufenoxuron (100, 50 or 31 mg a.i./L). The concentrations bioassayed corresponded to the minimum field-registered concentration (MiFRC), the half of that concentration (½MiFRC), and the median lethal concentration (LC_{50}) for *B. cockerelli* fourth-instar nymphs. The non-ionic surfactant Tween 20 was used at 0.01% (w/v) to enhance the adhesiveness of the insecticides. For the control, leaflets were dipped in distilled water plus surfactant only. Five replicates were used per concentration of each insecticide, plus a control.

After drying (~2 h after treatment), the tomato leaflets were individually placed in a cylindrical plastic tube (7.8 cm diameter × 10 cm height) that was open at both ends. The top of the cylinder was covered with a fine mesh screen to permit air circulation and to prevent the escape of the insects. Ten *E. varians* (5 females and 5 males, ≤5 d old) adults were placed into each cylinder using an aspirator. Eggs of *S. cerealella*, presented on a double-sided tape (1 cm^2) (Truper®, Jilotepec, Estado de México, Mexico) were supplied as food to *E. varians* adults. A 5% (w/v) solution of sugar (Chedraui®, Xalapa, Veracruz, Mexico) was also supplied to the predator in Eppendorf tubes (1.5 ml) with a piece of cotton and replaced every 3 d to prevent fungal growth. The petiole of the tomato leaflet was maintained in a nutritive solution to prevent dehydration. Adult mortality (distinguishing between males and females) was recorded daily for 3 d (72 h). After this time, the surviving adults were placed on non-treated tomato shoots to evaluate their longevity, predation, fecundity, as well as the nymphal development of first generation (F_1), as described later in the text. Adults were considered dead when they were gently touched with a fine brush and did not walk, jump, or fly.

Longevity, predation, fecundity, and nymphal development

Tomato shoots (~15 cm length) with one fully expanded true leaf were used in this test. All of the leaflets, except for the tip two leaflets, were removed from each leaf. These leaflets bearing a minimum of 16 and a maximum of 28 eggs + a mixture of 40 second- and third-instar (N_2-N_3; ≤ 8 h after ecdysis) of *B. cockerelli*. The tomato shoots were individually enclosed into a cylindrical plastic tube (8.5 cm diameter × 14.5 cm height), open at both ends. To maintain turgor, the tomato shoot was placed in the nutritive solution of Steiner (1984). One female of *E. varians* (8 days old), surviving to each concentration and insecticide of the bioassay described above, was randomly chosen and individually placed in this cylindrical plastic tube. The top of the cylinder was covered with a fine mesh screen. The predator female was transferred, each 72 h (= exposition) until dead, to an insect-free cylindrical plastic tube with a new tomato shoot with two leaflets similarly infested with eggs and nymphs N_2-N_3 as described above. Ten replicates for concentration, insecticide, and control were realized.

Longevity of *E. varians* females, trough to check them every 24 h until death, as well as the number of eggs and nymphs N_2-N_3 of *B. cockerelli* consumed over its lifetime was determined. Consumed eggs were easily distinguished because they looked dehydrated and no more yolk was left in them. In the case of the *B. cockerelli* nymphs that has been preyed upon, they were distinguishable because no more haemolymph was left in the body and because of the presence of a little brown spot at their dorsum, indicating the place where the predator inserted its stylet for feeding.

Fecundity was also determined. Several predator mirid species, among them *E. varians*, insert their eggs in the stems of the plant, petioles and main veins of the leaves (Pineda et al. 2016). Therefore, in order to compare fecundity among concentrations and insecticides, the number of nymphs of the F_1 per female, daily and cumulative, was recorded over the entire lifecycle of *E. varians*, as in Silva et al. (2016) and Mena-Mociño (2016) for this same predator species.

After emergence, between 35-56 nymphs of the F_1 generation that emerged from the third and fourth exposition in each concentration, insecticide, and control were individually placed in a Petri dish (9 cm in diameter × 1.5 cm in height) together with a tomato leaflet plus *S. cerealella* eggs as food. The petiole of the tomato leaflets were wrapped in a piece of moist cotton to prevent dehydration. The food was replaced every two days until nymphs completed their development. Each Petri dish was examined at 24-h intervals to determine when the nymphs moulted. From the number and the timing of moults, the number and duration of the nymphal instars were determined. The experiments were conducted in a controlled environment chamber at 25 ± 2°C, 60 ± 10% relative humidity, and a photoperiod of 16:8 h (L:D).

Data analysis

The statistical analysis of insect mortality was done by a two-way ANOVA, considering insecticide treatment as a factor and time (24, 48, and 72 h) as a second factor. The sex of the insect was initially evaluated as a third factor, but then dropped from the analysis because in general no significant differences between sexes were found (with a few exceptions detailed in the Results section). The longevity, predation, and fecundity of *E. varians* surviving females and the nymphal instars duration of the F_1 generation were analyzed by a one-way ANOVA considering insecticide treatment as a factor. All statistical tests were performed using SAS/STAT (version 9.4; SAS Institute, Cary, NC), the separate means were with the least significant difference (LSD) multiple range test ($p < 0.05$). Before analysis, data were checked for normality and homoscedasticity. All data are expressed as the mean ± SE.

Results

Adult mortality

When taking into account the sex factor, the mortality caused by the three concentrations of bifenthrin, abamectin, and imidacloprid on *E. varians* males and females comprised between 0-88% and 0-76%, respectively, at 24 h post-treatment (data not shown). Nevertheless, at 48 and 72 h post-treatment, this mortality was between 0-100% in both sexes for the three concentrations of these three insecticides. Only the ½MiFRC and MiFRC of abamectin at 24 h post-treatment ($F_{25, 104} = 11.07$; $P = 0.001$), as well as the MiFRC of bifenthrin at 48 h post-treatment ($F_{25, 104} = 29,38$; $P = 0.001$), caused significantly more mortality in females (between 44 and 48% for these three concentrations) than in males (between 16 and 24% for these three concentrations). Flufenoxuron did not cause any mortality at 24 and 48 h post-treatment in any of the three concentrations in either *E. varians* males or females, but at 72 h post-treatment a minimal mortality was observed for both sexes of the predator (4%) in the MiFRC. In the case of the control, mortality was not recorded in any of the three times evaluated. Because of this, the mortality data of males and females were analyzed jointly and it is reported as only adult mortality (Table 1).

The mortality of *E. varians* adults caused by the ½MiFRC and the MiFRC of abamectin (32 and 40%, respectively) and imidacloprid (50 and 82%, respectively) was significantly ($F_{12, 47} = 18.14$; $P = 0.001$) greater at 24 h post-treatment compared with that caused by the three concentrations of bifenthrin, flufenoxuron, and the control, as well as that recorded in the LC_{50} of abamectin and of imidacloprid (ranging between 2-17%, considering all the insecticides) (Table 1). The cumulative mortality caused by the ½MiFRC and MiFRC of bifenthrin, abamectin, and imidacloprid was significantly greater at 48 (between 30- 98%) ($F_{12, 50} = 72.62$ $P = 0.001$) and 72 h post-treatment (between 44 and 100%) ($F_{12, 51} = 101.47$; $P = 0.001$) with respect to that caused by the LC_{50} of each one of these insecticides (between

2-14% and 2-18% at 48 and 72 h post-treatment, respectively). Flufenoxuron caused no effect at 24 nor at 48 h post-treatment, and at 72 h post-treatment it only caused 4% of mortality (Table 1).

Table 1. Percentage of adult mortality (± SE) and longevity (days) of surviving *Engytatus varians* females (± SE) exposed to tomato leaflets treated with four insecticides.

Treatment	Concentration mg a.i./L	Mortality (%)			Longevity of females (days)
		24 h	48 h	72 h	
Control	**0**	0 (0)a	0 (0)a	0 (0)a	22.9 (1.41)c
Flufenoxuron	**LC_{50}**	0 (0)a	0 (0)a	0 (0)a	22.0 (1.85)bc
	½MiFRC	0 (0)a	0 (0)a	0 (0)a	22.0 (1.75)bc
	MiFRC	0 (0)a	0 (0)a	4.0 (2.44)a	20.3 (1.77)bc
Bifentrhin	**LC_{50}**	2.0 (2.00)a	4.0 (2.44)ab	8.0 (2.00)ab	22.1 (2.55)bc
	½MiFRC	17.0 (4.78)ab	30.0 (6.32)c	44.0 (6.78)c	20.3 (2.20)bc
	MiFRC	13.0 (3.33)ab	53.0 (13.33)d	72.0 (15.47)d	17.1 (2.42)ab
Imidacloprid	**LC_{50}**	8.0 (3.74)a	14.0 (6.00)b	18.0 (6.63)b	19.9 (1.71)bc
	½MiFRC	50.0 (16.43)d	78.0 (7.34)e	98.0 (2.00)e	ND
	MiFRC	82.0 (2.00)e	98.0 (2.00)f	100.0 (0)e	ND
Abamectin	**LC_{50}**	2.0 (2.00)a	2.0 (2.00)ab	2.0 (2.00)a	13.5 (1.61)a
	½MiFRC	32.0 (4.89)bc	88.0 (4.89)ef	100.0 (0)e	ND
	MiFRC	40.0 (10.80)cd	84.0 (6.78)e	100.0 (0)e	ND

Means within columns, for each evaluated time, followed by the same letter do not differ significantly (ANOVA, mean separation by LSD test, $P \leq 0.05$).

ND, Not determined because 100% mortality was observed for *Engytatus varians* adults.

Longevity of females

The longevity of the surviving *E. varians* females was only significantly affected by the MiFRC of bifenthrin (17 days) and the LC_{50} of abamectin (14 days) ($F_{8, 72} = 2.44$; $P = 0.021$), compared with the control treatment (23 days) (Table 1). In the remaining treatments, the longevity of the females was very similar (between 20 and 22 days) and did not differ from that recorded in the control.

Predation of females

All the insecticides affected the predation capacity of the surviving *E. varians* females (Table 2). The number of *B. cockerelli* eggs consumed/female was ≤10 during its entire life and it was significantly lower ($F_{8, 79} = 33.48$; $P = 0.001$) compared with the consumption recorded for the control females (24 eggs/female) (Table 2).

Table 2. Number of eggs and nymphs N_2-N_3 of *Bactericera cockerelli* consumed (± SE) by surviving *Engytatus varians* females exposed to tomato leaflets treated with four insecticides.

Treatment	**Concentration mg a.i./L**	**Consumption/female**	
		Eggs	**Nymphs**
Control	**0**	23.8 (2.05)f	277.1 (17.75)c
Flufenoxuron	**LC_{50}**	9.9 (1.35)d	254.0 (21.25)bc
	½MiFRC	8.9 (1.01)de	249.0 (16.82)bc
	MiFRC	5.2 (0.86)bc	228.0 (16.73)bc
Bifentrhin	**LC_{50}**	6.9 (1.05)ce	220.6 (31.84)bc
	½MiFRC	2.1 (0.60)ab	248.3 (24.84)bc
	MiFRC	0.5 (0.29)a	196.8 (26.30)ab
Imidacloprid	**LC_{50}**	9.1 (1.56)de	227.2 (18.42)bc
	½MiFRC	ND	ND
	MiFRC	ND	ND
Abamectin	**LC_{50}**	3.4 (0.50)abc	161.4 (16.42)a
	½MiFRC	ND	ND
	MiFRC	ND	ND

Means within columns followed by the same letter do not differ significantly (ANOVA, mean separation by LSD test, $P \leq 0.05$).

ND, Not determined because 100% mortality was observed for *Engytatus varians* adults.

The *E. varians* females that survived to the LC_{50} of abamectin and the MiFRC of bifenthrin treatments preyed on significantly ($F_{8, 75} = 2.43$; $P = 0.021$) fewer N_2-N_3 nymphs of *B.*

cockerelli (161 and 197 nymphs/female, respectively) during their entire life, compared with the control (277 nymphs/female) (Table 2). In the rest of the treatments, predation was variable (between 221 and 254 nymphs/female), but without significant differences with respect to the control (Table 2).

Fecundity

The total number of nymphs/female of *E. varians* obtained during its entire life was significantly lower ($F_{8,\,70} = 16.62$; $P = 0.001$) in the three concentrations of bifenthrin and flufenoxuron, as well as in the LC_{50} of abamectin and imidacloprid (between 5 and 59 nymphs/female), compared with that obtained in the control (79 nymphs/female) (Table 3).

Table 3. Number of nymphs per surviving *Engytatus varians* females (± SE) exposed to tomato leaflets treated with four insecticides.

Treatment	**Concentration mg a.i./L**	**Nymphs/female**	
		Per day	**Cumulative in their entire life**
Control	**0**	3.2 (0.32)bc	78.8 (6.0)c
Flufenoxuron	**LC_{50}**	0.8 (0.19)a	19.7 (6.62)a
	½MiFRC	0.6 (0.15)a	16.6 (4.03)a
	MiFRC	0.2 (0.08)a	4.8 (1.93)a
Bifentrhin	**LC_{50}**	2.4 (0.20)b	43.4 (6.67)b
	½MiFRC	2.5 (0.19)b	51.0 (5.96)b
	MiFRC	2.8 (0.35)b	41.7 (6.24)b
Imidacloprid	**LC_{50}**	2.7 (0.25)b	58.6 (6.95)b
	½MiFRC	ND	ND
	MiFRC	ND	ND
Abamectin	**LC_{50}**	3.8 (0.41)c	55.7 (6.42)b
	½MiFRC	ND	ND
	MiFRC	ND	ND

Means within columns followed by the same letter do not differ significantly (ANOVA, mean separation by LSD test, $P \leq 0.05$).

ND, Not determined because 100% mortality was observed for *Engytatus varians* adults.

On the other hand, the average number of nymphs/*E. varians* female per day was significantly lower ($F_{8,\ 70} = 27$; $P = 0.001$) only in the three concentrations of flufenoxuron (≤1 nymph/female), compared with the control (Table 3).

Number and duration of the nymphal instars of F_1 generation

The number of nymphs of the F_1 generation, originating from the *E. varians* females that survived to the treatments with the four bioassayed insecticides, was not affected. In all the treatments, five nymphal instars were recorded, the same as in the control. Nevertheless, the four insecticides significantly affected ($F_{28,\ 1652} = 13.71$; $P = 0.001$) the duration of these five nymphal instars. The duration of the nymphs of the first, second, and third instars comprised between 2.0-2.6, 2.1-2.9 and 2.2-3.0 days, respectively, except in the LC_{50} of abamectin where the nymphs of the first and second instar reached a duration of 4.6 and 4.0 days, respectively (Table 4).

The duration of the first instar was shorter in the treatments with the LC_{50} and MiFRC of bifenthrin (2.1 and 2.0 d days, respectively), while the LC_{50} of abamectin lengthened the duration of this nymphal instar to 4.6 days. In these cases, the averages were significantly different from those recorded in the control (2.4 days) (Table 4). For the second instar, the LC_{50} of flufenoxuron, imidacloprid, and abamectin, as well as the ½MiFRC of flufenoxuron, significantly lengthened its duration between 2.8 to 4 days with respect to the control (2.4 days), while the ½MiFRC of bifenthrin significantly reduced the duration of this nymphal instar to 2.1 days with respect to the control (Table 4). For the third nymphal instar, only the ½MiFRC of bifenthrin (2.2 days) reduced the duration with respect to the control (2.7 days). Moreover, the LC_{50} of imidacloprid (2.7 days) significantly reduced the fourth instar's development, compared with the control (3.1 days) (Table 4). The three concentrations of bifenthrin and the two of flufenoxuron (LC_{50} and the ½MiFRC) caused the nymphs of the fifth instar to live longer (between 4.2 and 4.4 days) than those of the control (3.8 days).

Table 4. Duration (days ± SE) of each nymphal instars, and total nymphal development, of the F_1 generation derived from surviving *Engytatus varians* females exposed to tomato leaflets treated with four insecticides.

Treatment	**Concentration mg a.i./L**	**n**	**Nymphal instar (d)**					**Total duration**
			N_1	**N_2**	**N_3**	**N_4**	**N_5**	
Control	**0**	**56**	2.4 (0.07)c	2.4 (0.07)b	2.7 (0.06)bc	3.1 (0.07)b	3.8 (0.09)a	14.6 (0.18)a
Flufenoxuron	**LC_{50}**	**52**	2.5 (0.06)c	2.8 (0.08)cd	3.0 (0.09)c	3.2 (0.09)b	4.4 (0.12)c	16.0 (0.21)b
	½MiFRC	**49**	2.6 (0.10)c	2.9 (0.12)d	2.8 (0.09)bc	3.0 (0.14)ab	4.3 (0.16)c	15.9 (0.33)b
	MiFRC		--	--	--	--	--	--
Bifentrhin	**LC_{50}**	**46**	2.1 (0.07)ab	2.3 (0.10)ab	2.5 (0.10)b	3.0 (0.11)ab	4.3 (0.11)c	14.4 (0.21)a
	½MiFRC	**56**	2.0 (0.00)a	2.1 (0.06)a	2.2 (0.06)a	3.2 (0.11)b	4.4 (0.09)c	14.0 (0.16)a
	MiFRC	**35**	2.4 (0.12)bc	2.5 (0.11)bc	2.5 (0.12)ab	3.0 (0.14)ab	4.2 (0.16)bc	14.5 (0.27)a
Imidacloprid	**LC_{50}**	**56**	2.6 (0.07)c	2.8 (0.10)cd	3.0 (0.09)c	2.7 (0.14)a	3.7 (0.14)a	14.6 (0.27)a
	½MiFRC		ND	ND	ND	ND	ND	ND
	MiFRC		ND	ND	ND	ND	ND	ND
Abamectin	**LC_{50}**	**53**	4.6 (0.21)d	4.0 (0.23)e	2.7 (0.14)bc	3.0 (0.16)ab	3.9 (0.17)ab	15.3 (0.65)ab
	½MiFRC		ND	ND	ND	ND	ND	ND
	MiFRC		ND	ND	ND	ND	ND	ND

Means within columns followed by the same letter do not differ significantly (ANOVA, mean separation by LSD test, $P \leq 0.05$).

ND, Not determined because 100% mortality was observed for *Engytatus varians* adults.

--, Not determined because only four nymphs emerged.

In the case of the LC_{50} of imidacloprid (3.7 days) and of abamectin (3.9 days), the development times were not significantly different from those obtained in the control (Table 4). In relation to the total time of development, significant differences were observed ($F_{7, 308}$ = 6.88; P = 0.001) when the nymphs originated from *E. varians* females that had survived the LC_{50} and the ½MiFRC of flufenoxuron (16 and 15.9 days, respectively), as compared with the rest of the treatments (between 14.0-15.3 days) and the control (14.6 days) (Table 4). The total time of development was not determined in the nymphs originating from *E. varians* females exposed to the MiFRC of flufenoxuron because only four nymphs emerged.

Discussion

Since the first report in Mexico of the presence of *E. varians* (Martínez el al. 2014), few studies have been conducted to evaluate the effects that chemical insecticides used for the control of *B. cockerelli* in tomato crops can cause on this predator. In the present study, the ½MiFRC and MiFRC of the three neurotoxic insecticides tested (imidacloprid, abamectin, and bifenthrin) caused high cumulative mortality at 72 h post-treatment (between 72-100%, except ½MiFRC [44%]) on the *E. varians* adults when they were exposed to tomato leaflets treated with these compounds. The high toxicity of these compounds, or others that belong to the same chemical group, for mirid predators has already been recognized previously when the insects were treated through different application methods. In this respect, and similar to the results obtained in the present study, Pérez-Aguilar et al. (2018) reported that, at 72 h post-treatment, imidacloprid, thiamethoxam (another neonicotinoid), and abamectin caused between 78-95% of mortality of *E. varians* adults when were exposed to the maximum recommended field concentrations for the pest control in the tomato crop in Brazil. Similarly, the mortality of the *Macrolophus basicornis* (Stäl) (Wanumen et al. 2016a, Passos et al. 2017), *N. tenuis* (Ziaei et al. 2017), and *Deraeocoris brevis* (Uhler) (Amarasekare and Shearer 2013) adults was $\geq$90% when were exposed to tomato leaflets treated with abamectin, imidacloprid or with the pyrethroids cypermethrin and lambda-cyhalothrin under laboratory, extended laboratory or greenhouse conditions. There could be two reasons for the high mortality caused by these insecticides, as well as that obtained in the present study. First, the insecticides that belong to these chemical groups, among them imidacloprid, abamectin, and bifenthrin, act by contact (Gunning and Moores 2001; Lasota and Dybas 1991; Tewary et al. 2005). Therefore, the adults of these predators could have been contaminated through the tarsi (Mahdian et al. 2007; Biondi et al. 2012) with the residues of the insecticides present in the Petri dishes or in the tomato leaflets. Second, imidacloprid (Buchholz and Nauen 2001) and abamectin (Dembilio et al. 2014) have systemic properties. During the experiments of the present study, *E. varians* adults were fed with *S. cerealella* eggs. Thus, because of their zoophytophagous behavior they could also feed themselves with the sap of the tomato leaflets contaminated by the insecticides. In this case, possibly the individuals received a higher quantity of the compounds compared with those that were only in contact with the residues present in the vegetal tissue surface, as was the case of *N. tenuis* and *D. brevis* that were fed with *E. kuehniella* eggs treated with cypermethrin, deltamethrin or lambda-cyhalothrin (Amarasekare and Shearer 2013; Wanumen et al. 2016b; Ziaei et al. 2017).

Flufenoxuron was the insecticide that affected the *E. varians* adults the least (4% of mortality in the highest concentration). The low mortality caused by this compound could be expected given that the chitin biosynthesis inhibitor, such as flufenoxuron, mainly act through ingestion on the immature stages of the insects by inhibiting the formation of chitin biosynthesis in the molting process (Merzendorfer 2013; Sun et al. 2015). In this respect, flufenoxuron and teflubenzuron caused 66 and 45% of the cumulative mortality on third instar of *E. varians* (Pérez-Aguilar et al. 2018) and *M. basicornis* (Passos et al. 2017) at 3 and 9 days post-treatment, respectively. Flufenoxuron, lufenuron, and novaluron, however, caused 100% mortality on nymphs of the first instar of *Pilophorus typicus* (Distant) (Hemiptera: Miridae) at 120 h post-treatment (Nakahira et al. 2010). On the other hand, photolysis could also contribute to the low adult mortality of *E. varians* that was recorded in the present study. In this sense, Sun et al. (2015) reported that natural or artificial light is one of the main factors in rapidly breaking down the chitin synthesis inhibitors into inactive materials. Nevertheless, in contrast with our results, several studies have reported that other chitin synthesis inhibitors, including flufenoxuron, caused a more significant effect on the adult state of some species of mirid predators, including *E. varians*. In this regard, teflubenzuron, novaluron, and flufenoxuron caused between 8-24% of mortality on *M. basicornis* (Passos et al. 2017), *D. brevis* (Amarasekare and Shearer 2013), and *P. typicus* (Nakahira et al. 2010) adults or of the species used in the present study (Pérez-Aguilar et al. 2018). This variability in the mortality could be explained by the different methodologies used, the individuals' length of time of exposure to the treatments (3, 10 or 12 days), the methods of application of the insecticides (residual, topical or ingestion) or the substrates used (Petri dishes, tomato or kalanchoe [*Kalanchoe laciniata* Adans; Crassulaceae] leaves).

The sublethal effects related to longevity, predation, fecundity, and nymphal development time were not evaluated with the *E. varians* adults exposed to the ½MiFRC and MiFRC of abamectin and imidacloprid because these concentrations caused 100% mortality on the treated adults. Nevertheless, the MiFRC of bifenthrin and the LC_{50} of abamectin reduced significantly 1.33 and 1.70 times the longevity of the surviving *E. varians* females, respectively. In another study, the pyrethroid lambda-cyhalothrin also reduced the longevity of the *D. brevis* females by 5 and 18 times when treated with 5 and 49.9 mg a.i./L, respectively, compared with the control (37 days) (Amarasekare and Shearer 2013). Conversely, none of the three concentrations of flufenoxuron affected the longevity (between 20-22 days *vs* 23 days for the control) of the *E. varians* females. Similarly, the longevity of the females of this same predator was not affected when were exposed in Petri dishes treated with 3.75 mg a.i./L of teflubenzuron (27 days *vs* 32 days in the control) or when they were originated from third instar nymphs treated with the same compound (23 days *vs* 29 days in the control; Pérez-Aguilar et al. 2018). In contrast, 38.9 and 388.5 mg a.i./L of novaluron, reduced 1.3 and 2.1 times, respectively, with respect to the control (37 days), the longevity of *D. brevis* females fed with *E. kuehniella* eggs, exposed to contaminated glass cylinder or when were topically treated with this compound.

Under laboratory conditions, and in a no-choice experiment, an *E. varians* female that had not been exposed to any treatments with insecticides preyed on 40 eggs, as well as 16 nymphs N_2 and 14 nymphs N_3 of *B. cockerelli* during 24 h (S. Pineda, unpublished data). The only other mirid species where the predation of eggs of this pest has been recorded is *D. hesperus*. Also, in a no-choice experiment, the females of this predator consumed 5 eggs of *B. cockerelli* during the same period of time mentioned (Ramírez-Ahuja et al. 2017). Nevertheless, in the present study, where *E. varians* females that survived the treatments with imidacloprid, abamectin, bifenthrin, and flufenoxuron had eggs or a mixture of N_2-N_3 *B cockerelli* nymphs available, they consumed between 2.5-48 times fewer eggs than those of the control (24 eggs/female). Similarly, the consumption of a mixture of N_2-N_3 nymphs of *B.*

cockerelli/female of the predator was significantly reduced 1.4 and 1.7 times in the MiFRC and LC_{50} of bifenthrin and abamectin, respectively (277 N_2-N_3 nymphs/female in the control). Although the reasons why bifenthrin and abamectin decreased the predation capacity of *E. varians* are unknown, it is possible that these insecticides have modified some systems of the adults of this predator and as a consequence lessened their searching capacity and consuming (Martinou et al. 2014; Zhang et al. 2015). Similarly, the females of the mirid predator *Cyrtorhinus lividipennis* Reuter, exposed to 2 and 5 mg a.i./L of deltamethrin, consumed 1.6 times fewer nymphs of *Nephotettix lugens* Distant (Homoptera: Euscelidae) with respect to the control (5 nymphs/female) (Zhang et al. 2015); nevertheless, these authors did not specify the nymphal instar of the prey used.

It is well known that the longevity of insects is associated with their food sources (Burla et al. 2014; Silva et al. 2016), among other factors. Thus, the decrease in the predation by the *E. varians* females that were treated with the MiFRC of bifenthrin and the LC_{50} of abamectin, on a mixture of N_2-N_3 *B. cockerelli* nymphs can also explain the reduction in their longevity caused by these same concentrations of these compounds. Therefore, it is essential to point out that mortality is not the most critical factor to be considered as an end point in studies with insecticides regarding natural enemies. The adverse effects caused by bifenthrin and abamectin on the predation capacity and the longevity of *E. varians* females are very important from the practical point of view. Bifenthrin and abamectin are traditionally used for the control of *B. cockerelli* in solanaceous crops, where *E. varians* is found. Consequently, these compounds can affect the population dynamic and compromise the effectiveness of this predator as an agent of biological control.

Little information is available about the effects caused by chemical insecticides in the subsequent generations of the individuals of the treated insects. In the present study, only the LC_{50} of both imidacloprid and abamectin, as well as the three concentrations of bifenthrin, reduced between 1.3 and 1.9 times the fecundity of surviving *E. varians* females. The reason for this is not immediately apparent, but we speculate that such effects may be due to the costs of fighting the intoxication involving the transfer of energy reserves to combat the neurotoxic effect of these compounds. Similarly, a significant reduction in fecundity was observed when the females of the predator *Orius laevigatus* (Fieber) (Hemiptera:

Anthocoridae) were exposed to tomato leaflets treated with abamectin and emamectin benzoate (9 and 2.5 times, respectively; Biondi et al. 2012) or when the females of *O. insidiosus* (Say) (Hemiptera: Anthocoridae) were exposed to Petri dishes treated with bifenthrin, imidacloprid or thiamethoxam (between 1 and 1.45 times; Fernandes et al. 2016).

Flufenoxuron was the compound that drastically reduced the number of nymphs/*E. varians* females. In the same way, the fecundity of the females of the predator *Podisus nigrispinus* Say (Heteroptera: Pentatomidae) was significantly 2.5 times lower than in the control (460 nymphs/female) when were fed with *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) larvae that had previously been fed with soybean leaves (*Glycine max* L.) treated with 35 mg a.i./L of diflubenzuron (Castro et al. 2012). In the *E. varians* females, the number of nymphs/female was reduced 1.7 times when were treated as third instar with 3.75 mg a.i./L of teflubenzuron (Pérez-Aguilar et al. 2018). Even though it has been reported that chitin synthesis inhibitors are safe for non-target insects (Sun et al. 2015), their effects have been recorded on the reproductive processes of certain species of natural enemies. Medina et al. (2002) reported that diflubenzuron affected the fertility, but not the fecundity of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). The chitin synthesis inhibitors, like other insect growth regulators, can be transported through oogenesis to the ovaries of the treated females (Medina et al. 2002; Mansur et al. 2010). As a consequence, these compounds interrupt the maturation of the eggs in the ovaries or the embryonic development because they inhibit the formation of chitin in the tracheal system, in the epidermis, in the epithelium, and in the peritrophic membrane of the midgut (Muthukrishnan et al. 2012, Merzendorfer 2013). This last effect could occur in the present study, when the fecundity of the *E. varians* females was not determined by the number of laid eggs but by the number of nymphs/female. Thus, more studies are currently being performed to know the real impact of flufenoxuron on the fecundity of this important predator of *B. cockerelli*.

In the present study, none of the insecticides bioassayed affected the number of nymphal instars of the F_1 generation originating from the surviving females of *E. varians*. Nevertheless, these compounds significantly affected the duration of each nymphal instar, but a uniform pattern was no observed since in some cases the duration of some instars decreased and in others it increased. Also, only the LC_{50} and the ½MiFRC of flufenoxuron significantly lengthened (24 h) the nymphal development in the F_1 generation. Similarly, teflubenzuron lengthened the total time of development (nymph + adult) of the F_1 generation of *M. basicornis,* originating from fourth instar nymphs treated with this compound, by 5 days (45 *vs* 40 days in the control; Passos et al. 2018). Previously, Pineda et al. (2016) reported that the longevity of the *E. varians* females was 22 days. In this study, the nymphs that were used to evaluate the development time came from the third and fourth expositions, when the *E. varians* females were 17 and 20 days old, respectively, and were near death. Therefore, the effects on the development delay of *M. basicornis* reported by Passos et al. (2018) and those recorded in the current study caused by flufenoxuron and teflubenzuron probably are due to a reduction in the maternal investment per egg in later ages, as reported by Giron and Casas (2002). In their experiment with the parasitoid *Eupelmus vuilletti* (Crawford) (Hymenoptera: Eupelmidae) there was a reduction in egg's nutritional content throughout of mother's life, with possible effects on the progeny. Therefore, individuals from eggs with lower nutrient content took longer time to reach adulthood.

In conclusion, the lethal (mortality) and sublethal effects (longevity, predation, and fecundity) of the surviving *E. varians* females, as well as the nymphal development of the F_1 generation caused by imidacloprid, abamectin, and bifenthrin depended on the tested concentration. Therefore, it is necessary to design a plan regarding the use of these three compounds in areas where *E. varians* is naturally found or in deliberate releases. On the other hand, because flufenoxuron affected the fecundity and nymphal development time in *E. varians* more studies are needed under more real conditions to evaluate its compatibility with this predator before being included in an integrated management program for the control of *B. cockerelli*.

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5. DISCUSIÓN GENERAL

El conocimiento de los efectos negativos que ocasionan los insecticidas sobre los parámetros biológicos de los enemigos naturales se ha documentado durante las últimas tres décadas (Phillips et al. 1993; Viñuela et al. 1998; Tedeschi et al. 2001; Elzen 2001; Pérez-Aguilar et al. 2018; Morales et al 2019). En el caso de los enemigos naturales de *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae), la mayoría de los estudios se han enfocado al *efecto letal* que causan estos compuestos sobre el estado adulto (Luna-Cruz et al. 2011, 2015; Morales et al. 2018; Pérez-Aguilar et al. 2018). Sin embargo, en los estados inmaduros de los enemigos naturales, particularmente los parasitoides cuyos inmaduros están protegidos en el interior del huésped, así como en individuos de generaciones subsecuentes que provienen de insectos supervivientes a las aplicaciones de insecticidas, también pueden ser afectados en su orientación, parasitismo, depredación, desarrollo, reproducción y proporción de sexos (Stark y Banks 2003; Desneux et al. 2007), lo que se conoce como *efectos subletales*.

En el presente estudio se determinaron los efectos letales y subletales de los insecticidas aceite de soya, imidacloprid, abamectina, bifentrina, dimetoato, flufenoxuron o spinosad sobre los adultos o estados inmaduros del ectoparasitoide *Tamarixia triozae* (Hymenoptera: Eulophidae) y el depredador *Engytatus varians* (Hemiptera: Miridae). Estos insecticidas son comúnmente utilizados para el control de *B. cockerelli* sobre cultivos de solanáceas y pertenecen a diferentes grupos toxicológicos: aceites, neonicotinoides, avermectinas, piretroides, organofosforados, espinosinas y reguladores del crecimiento que inhiben la síntesis de quitina (Dávila-Medina et al. 2012; Cerna et al. 2015; Morales et al. 2018;). Los estados inmaduros de *T. triozae* fueron muy sensibles a la mayoría de los insecticidas evaluados. El aceite de soya, imidacloprid y abamectina alteraron el parasitismo y alimentación de las hembras de *T. triozae* cuando se trataron en las etapas de huevo, larva y pupa. Este efecto dependió del insecticida, la concentración sobre los estados inmaduros del parasitoide y sobre el ínstar de *B. cockerelli* ofrecido para parasitismo y alimentación. Similarmente, los insecticidas neonicotinoides tiametoxam e imidacloprid, y las avermectinas benzoato de emamectin y abamectina también afectaron el parasitismo de *Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae) sobre *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae) (Lombardi de Carvalho 2008). Como también sucedió con *Eretmocerus mundus* (Mercet) (Hymenoptera, Aphelinidae) (Fernández et al. 2010, 2015*;* Sohrabi et al. 2013) y *Encarsia inaron* (Walker) (Hymenoptera: Aphelinidae) (Sohrabi et al. 2012) sobre *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), cuando las hembras de estos parasitoides se trataron en los estados inmaduros de larva y pupa.

Al igual que el parasitismo, la alimentación sobre el huésped de los parasitoides sinovigénicos está reconocida como importante en la supresión de poblaciones de insectos plaga (Yang et al. 2012; Liu et al. 2015; Cheng et al. 2017). En el presente estudio, independientemente del estado inmaduro tratado (huevo, larva o pupa), el aceite refinado de soya, imidacloprid y abamectina no causaron una disminución en la alimentación del parasitoide sobre ninfas de cuarto y quinto ínstar del hospedero. En contraste, estos tres insecticidas, en general, alteraron la alimentación de las hembras de *T. triozae* sobre las ninfas de segundo y tercer ínstar de *B. cockerelli*. Debido a que las ninfas de segundo y tercer ínstar son muy importantes en la alimentación de *T. triozae* (Morales et al. 2014; Rojas et al. 2015; Ramírez-Ahuja et al. 2017), esta reducción en la alimentación del huésped podría comprometer la eficacia y la supervivencia del enemigo natural si no pueden obtener los nutrientes esenciales para el desarrollo de sus huevos.

Existen varios trabajos que aseguran efectos transgeneracionales desfavorables en la proporción sexual de himenópteros parasitoides, cuando éstos se expusieron a residuos de insecticidas en etapas de huevo-larva, pre-pupa o pupa (Biondi et al. 2013; Sohrabi et al. 2012; Abreu et al. 2014). Sin embargo, en este trabajo, y con los insecticidas aceite refinado de soya, imidacloprid y abamectina, no se modificó la proporción de sexos de *T. triozae*. Este hallazgo resulta favorable porque el establecimiento y prevalencia de una población, así como el éxito del control biológico es realizado por las hembras del parasitoide (Van Driesche et al. 2007; Rojas et al. 2015). No obstante, quizá deba ensayarse en condiciones más cercanas a la realidad para asegurar que no se causará daño a este enemigo natural.

Es bien sabido y aceptado que la presencia de los residuos de los insecticidas sobre el follaje de las plantas pueden causar efectos letales y subletales sobre los enemigos naturales y, en consecuencia, por supuesto interferir en el control biológico (Peshin y Zhang 2014; Quijano et al. 2016). En este trabajo se encontró que los residuos de spinosad, flufenoxuron, dimetoato e imidacloprid disminuyeron con el tiempo cuando se aplicaron sobre plantas de tomate en invernadero. Así mismo, se determinó que la mayor cantidad de degradación de estos compuestos ocurrió entre los 30 y 40 días post-aplicación. Las concentraciones de los residuos de espinosinas A y D, flufenoxuron y dimetoato disminuyeron con un tiempo medio de vida entre 34-40 días, mientras que la de imidacloprid fue de 120 días. La degradación de los insecticidas en las hojas de tomate puede estar influenciado por factores abióticos (condiciones climáticas, tipo de aplicación, dosis, intervalos de aplicación) y bióticos (especie de la planta y edad de la planta). No obstante, el tiempo que se detectaron residuos en las hojas de tomate debe considerarse con cuidado para medir los riesgos sobre los enemigos naturales en esta condición de invernadero.

Por otro lado, los adultos de *E. varians* fueron muy susceptibles a los residuos de imidacloprid y dimetoato, incluso 30 días post-aplicación de estos compuestos. En contraste, spinosad causó baja mortalidad (10% a los 0 y 10 días post-aplicación) y flufenoxuron no causó ningún efecto. Otros autores también encontraron alta toxicidad de imidacloprid y tiametoxam y otros organofosforados (clorpirifos, acefato y metamidofos) poco después de su aplicación (Van De Veire et al. 2002; Fernandes et al. 2016; Wanumen et al. 2016; Figuls et al. 1999) sobre los depredadores *Orius laevigatus* (Fieber), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), *Macrolophus basicornis* (Stäl), *Macrolophus caliginosus* (Wagner) y *Dicyphus tamaninii* Wagner (Hemiptera: Miridae), pero la actividad biológica de estos compuestos disminuyó durante los días siguientes. En contraste, spinosad y flufenoxuron fueron seguros para los adultos de *E. varians*, como se reportó para otras especies de heterópteros. Por ejemplo, *O. laevigatus* (Van De Veire et al. 2002), *Pilophoru typicus* (Distant) (Hemiptera: Miridae) (Nakahira et al. 2010), y *Macrolophus pygmaeus* (Rambur) y *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) (Arnó y Gabarra 2011).

En condiciones de laboratorio, los tres insecticidas neurotóxicos evaluados (imidacloprid, abamectina y bifentrina) causaron una alta mortalidad acumulada sobre los adultos de *E. varians,* cuando se expusieron a foliolos de tomate tratados con estos compuestos. En contraste, flufenoxuron fue el insecticida que menos afectó a los adultos de *E. varians*. Los efectos subletales de longevidad, depredación y fecundidad, así como el tiempo de desarrollo ninfal de la generación F_1 fueron afectados por alguna de las concentraciones de flufenoxuron, bifentrina, imidacloprid o abamectina sobre las hembras de *E. varians* sobrevivientes a los tratamientos. Abamectina y bifentrina fueron los únicos que redujeron la longevidad de las hembras sobrevivientes de *E. varians*, así como su depredación. Las causas de estos efectos no son claras; a manera de especulación, es posible que estos insecticidas causen algún daño en el sistema nervioso que disminuya su capacidad de búsqueda y consumo de su presa (Martinou et al. 2014; Zhang et al. 2015), lo que también puede explicar la disminución en la longevidad (Burla et al. 2014; Pineda et al. 2016; Silva et al. 2016).

Por otra parte, imidacloprid, abamectina y bifentrina redujeron la fecundidad de las hembras sobrevivientes de *E. varians*, pero este parámetro biológico fue más drásticamente afectado por flufenoxuron. La reducción de la fecundidad causada por insecticidas inhibidores de la síntesis de quitina también fue reportada en el pentatómido *P. nigrispinus* (Castro et al. 2012) y nuestra misma especie estudiada (Pérez-Aguilar et al. 2018) cuando se trataron con diflubenzuron y teflubenzuron, respectivamente. Imidacloprid, abamectina, bifentrina y flufenoxuron afectaron la duración de cada ínstar ninfal de *E. varians*; sin embargo, la duración de algunos ínstares disminuyó y en otros se prolongó y únicamente el desarrollo ninfal total fue afectado por flufenoxuron. Estos efectos sobre la fecundidad pudieran deberse a que interrunpen en la maduración de ovarios, huevos o el mismo desarrollo del embrión (Sun et al. 2015); no obstante, todas estas sospechas se deben corroborar con análisis histológicos y fisiológicos antes de aceptarlas. El retraso en el desarrollo ninfal de *E. varians* es probable que se deba a un bajo contenido de nutrientes presente en los huevos depositados por las hembras que fueron expuestas a residuos de los insecticidas, tal como sucedió en el zoofitófago *M. basicornis* (Passos et al. 2018), necesitando más tiempo para llegar a la etapa adulta.

La aportación del presente estudio debe complementarse con estudios en condiciones más reales para evaluar los efectos subletales y los riesgos que pueden representar para la inclusión de alguna de esas herramientas químicas de control en el Manejo Integrado de *B. cockerelli*. Sin embargo, el riesgo que conlleva el uso de *T. triozae* y *E. varians* en ambientes contaminados por insecticidas, puede comprometer el éxito de ambos enemigos naturales en el control biológico de *B. cockerelli*, así como su prevalencia en el cultivo de tomate.

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6. CONCLUSIONES GENERALES

- El insecticida derivado del aceite refinado de soya, así como imidacloprid, abamectina, dimetoato, bifentrina, spinosad y/o flufenoxuron afectaron el parasitismo y alimentación sobre el huésped de las hembras de *T. triozae*. Así mismo, cuando los adultos del depredador zoofitófago *E. varians* se expusieron a los residuos de estos compuestos, presentes en los foliolos de tomate en laboratorio e invernadero, causaron mortalidad y efectos sobre la longevidad, tasa de depredación, fecundidad y el tiempo de desarrollo ninfal
- Imidacloprid, abamectina y el aceite refinado de soya afectaron el parasitismo y alimentación de las hembras de *T. triozae* cuando se trataron en los estados inmaduros de huevo, larva y pupa. Sin embargo, independientemente del estado inmaduro del parasitoide tratado, el parasitismo prevaleció en ninfas de mayor tamaño (tercero, cuarto y quinto instar) de *B. cockerelli*, mientras que la alimentación generalmente disminuyó con el tamaño de su hospedero (quinto < cuarto < tercero < segundo ínstar). La disminución en la alimentación del segundo y tercer ínstar puede ser particularmente riesgoso para la biología del parasitoides, porque son los ínstares que prefiere para alimentarse del huésped.
- Dimetoato e imidacloprid fueron altamente nocivos para los adultos de *E. varians,* aún a los 30 días post-aplicación. Por lo tanto, estos insecticidas deben ser usados con precaución en áreas donde este enemigo natural esté presente naturalmente, o en áreas donde se libere. En contraste, spinosad y flufenoxuron fueron inocuos para los adultos de este depredador, debido a ello probablemente estos compuestos pueden integrarse en un programa de MIP, al menos sobre cultivos de tomate en condiciones de invernadero donde este depredador ha demostrado su potencial como un agente de control biológico contra *B. cockerelli*.
- En condiciones de laboratorio, imidacloprid, abamectina y bifentrina causaron efectos letales sobre los adultos de *E. varians*, con excepción de flufenoxuron, como también se demostró en condiciones de invernadero. Las tasas de longevidad, depredación y fecundidad de las hembras de *E. varians* sobrevivientes a las aplicaciones de los insecticidas, así como el desarrollo ninfal de la generación F_1, dependieron de la concentración de los productos y de sus grupos toxicológicos.