UNIVERSITAT
POLITÈCNICA
DE VALÈNCIA

ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRONÓMICA Y DEL MEDIO NATURAL

Bases para la gestión integrada de *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) en cítricos

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Directores: Dr. Alberto Urbaneja García - Dr.Alejandro Tena Barreda

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Tesis Doctoral

Laura Planes Insa

Directores: Dr. Alberto Urbaneja García
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Valencia, Enero 2016

“A veces encontramos nuestro destino
en el camino que tomamos para evitarlo”

Kung Fu Panda

Agraïments

Mirant enrere veig moltes persones que han posat el seu granet per poder realitzar aquesta tesi.

En primer lloc agrair als meus directors, el Dr. Alberto Urbaneja i el Dr. Alejandro Tena, l'oportunitat que m'han donat de conèixer aquest "petit món". Una àrea que no vaig pensar que m'anava a interessar i on no vaig creure que podria treballar. La vostra passió per l'entomologia la trasmitiu de tal manera que tot sembla més fàcil. Gràcies pels vostres consells, paciència i suport.

A Ramon Aparici i Alberto García del Insectari de la Generalitat Valenciana a Almassora i a Koppert Biological Systems pel subministrament dels insectes necessaris per a la realització d'aquest treball.

El meu agraïment a Batista i Bernardoper permetre realitzar els assaigs en les seues parcel·les i per la seua ajuda en els tractaments.

A Helga, Josep i Pili que em van ensenyar, amb més i amb menys paciència, l'entrellat dels assajos i les seues tantes repeticions.

A tot Departament d'Entomologia de l'IVIA i de l'UJI, un equip humà amb qui els dies de camp i laboratori s'han fet més lleugers. Quànta gent hi ha darrere d'un assaig! Als que estaven quan vaig arribar i als que van arribar quan em vaig anar: a Josep Jacas,Alfons, Beatriz, César, Consuelo, Cristian, Elena, Francesc, Jesús, Joel, Luis, María, Mª Jesús, Mª José, Marian, Manu, Miquel, Molli, Mónica, Nacho, Óscar D., Pablo B., Pablo U., Paco, Poliane, Ahlem, Amparo, Azucena, Ruth, Tati, Tina, Vicky, ...

A Patricia Chueca porque, aunque no hemos estado en el mismo departamento, siempre has estado dispuesta echar una mano con cualquier problemilla, a animarme cuando las cosas no salían y porque, a veces, una buena "xarraeta" es lo único que se necesita para levantar el ánimo.

A Mercedes i Miguel por hacerme sentir una más.

Als meus pares, a Aina, Pere, Lola i Llorenç que sempre esteu allí o ací, on faja falta.

A Javi, que me haces ver el lado guasón de la vida (seguro que estás contento de que esta tesis llegue a su fin).

I als meus xics, a Marc i Jordi un huracà de felicitat aquest últim any.

Summary

Pezothrips kellyanus (Bagnall) (Thysanoptera: Thripidae) is a new citrus pest in the Mediterranean basin. This thrips species was described in Australia in the last century. In Spain, the first damages were observed in the locality of Alzira, Valencia, in 2007. Currently, it is widely distributed in the Valencia region and it is considered a key pest. Nymphs of *P. kellyanus* refuge and feed on the surface of young fruitlets. This feeding habit causes patches or rings of tissue scar around the apex as fruit mature. Although this thrips species does not affect the internal quality of the fruit, such damage leads to economic losses because of the reduced market value of the affected fruit. Up to 100% of fruit can be damaged.

Despite the worldwide distribution and economic importance of *P. kellyanus*, its biological control is still under development and chemical control is the only alternative for growers. However, its implementation, results, and side effects are poorly known. In order to improve the integrated management of *P. kellyanus* we determined the seasonal trend of *P. kellyanus* nymphs during the period in which the young fruitlets are sensitive to thrips damage. Moreover, we studied the diurnal distribution of first and second generation *P. kellyanus* nymphs, as well as, the spatial distribution within the tree of the nymphs and damages. These results will improve the sampling protocols and the insecticide applications. The seasonal trend (number of generations attacking the fruit) and damages of *P. kellyanus* nymphs depended on the orchards and years. This result emphasizes the importance of sampling weekly from petal until six weeks later. Furthermore, according to the data obtained, when there is a second generation of *P. kellyanus* nymphs, this is generally more damaging.

Pezothrips kellyanus nymphs showed a clear preference for fruit located at the top of the trees, which coincided with the highest percentage of damaged fruit in this area. On the other hand*,* *P. kellyanus* nymphs were uniformly distributed among the four cardinal directions of the canopy and throughout the day. Therefore, the cardinal directions and time of day does not seem to be an important factor to develop a sampling plan or to spray insecticides. In this same study, it was found that the fruits occupied by *P. kellyanus* nymphs tend to fall in a higher proportion than those that were not occupied by nymphs.

The next objective of this thesis was to determine the efficacy of three insecticides (chlorpyrifos, spinosad and spirotetramat) to control *P. kellyanus* nymphs. These insecticides were selected because of their different mode of action. Chlorpyrifos and spinosad showed a high efficacy against *P.* kellyanus nymphs. They

significantly reduced the percentage of damaged fruit when there was one generation of nymphs. However, their persistence was not enough to prevent the attack of a second generation of *P. kellyanus* nymphs. Spirotetramat had not a shock effect against this pest and it could not avoid the attack of a second generation. Finally, in this field work, we analyzed the side effects of these three insecticides on natural enemies present at the time of the treatment. Spinosad as spirotetramat negatively affected phytoseiids. Due to low populations of other natural enemies we could not assess the side effects on them. Therefore, we decided to study the side effects of these insecticides on coccinelids and parasitoids (key natural enemies in citrus) under laboratory conditions.

First, lethal and sublethal side effects of spirotetramat (as the lethal and sublethal effects of chlorpyrifos and spinosad were already known) on adults and larvae of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) were evaluated under laboratory conditions by topical application and by ingestion of treated individuals of *Planococcus citri* Risso (Hemiptera: Pseudococcidae). Spirotetramat resulted harmless when directly applied on larvae and adults of *C. montrouzieri*, since it did not affect survival, longevity, fecundity, egg hatching, and offspring survival. When larvae and adults of *C. Montrouzieri* were fed with treated prey, spirotetramat was also classified as harmless.

Second, we studied lethal and sublethal effects of spirotetramat, spinosad and chlorpyrifos on *Aphytis melinus* DeBach (Aphelinidae Hymenoptera), the main parasitoid of California red scale, *Aonidiella aurantii* (Maskell) (Hemipteta: Diaspididae). For *A. melinus* adults, chlorpyrifos and spinosad were classified as toxic but spirotetramat resulted moderately toxic. For immature (larvae), chlorpyrifos was moderately toxic, spirotetramat was slightly toxic and spinosad resulted harmless. Takin into consideration these results, spirotetramat could be used against *P. kellyanus* in orchards where population levels are low and an application against *A. aurantii* is also need because this insecticide shows high efficacy against this pest.

Resumen

Pezothrips kellyanus (Bagnall) (Thysanoptera: Thripidae) es una nueva plaga de cítricos en el Mediterráneo que fue descrita por primera vez en Australia en el siglo pasado. En 2007 se observaron los primeros daños en nuestros cítricos, concretamente en Alzira, en la comarca de la Ribera Alta, siendo actualmente, una plaga más de los cítricos valencianos. Las ninfas de *P. kellyanus* se refugian y alimentan de la superficie de los frutos recién cuajados produciendo unas escarificaciones circulares alrededor del pedúnculo. Los daños son meramente estéticos y devalúan su valor en el mercado, pudiendo alcanzar el 100% de frutos dañados.

Hoy en día, el control químico es prácticamente la única alternativa contra este trips, cuyos daños son muy variables en función de la variedad, la zona y también los años. Para poder mejorar la gestión integrada de *P. kellyanus* es necesario un mayor conocimiento sobre su biología y ecología en campo. Para ello, se ha seguido la dinámica poblacional de las ninfas de *P. kellyanus* durante el periodo en que los frutos recién cuajados son más sensibles a los daños producidos por las ninfas. Además, se ha estudiado la distribución de la plaga dentro de los árboles y su movimiento a lo largo del día, factores necesarios tanto para estimar la población de trips como para afinar las aplicaciones de productos fitosanitarios. Tanto la dinámica como los daños variaron según parcelas y años. Tras la caída de pétalos se observaron una o dos generaciones de ninfas que produjeron daños en los frutos. Este resultado subraya la necesidad de realizar muestreos semanales desde la caída de pétalos y continuarlos incluso después del tratamiento contra la primera generación. Además, según los datos obtenidos, cuando se da una segunda generación de *P. kellyanus*, ésta es más dañina para los frutos.

Las ninfas de *P. kellyanus* mostraron una clara preferencia por los frutos situados en la parte alta de la copa, lo que coincidió con el mayor número de frutos dañados en esta zona. Sin embargo, la distribución de las ninfas fue uniforme en las cuatro orientaciones del árbol y su abundancia tampoco varió a lo largo del día. Por lo tanto, la orientación y el momento del día no parecen ser claves a la hora de desarrollar un plan de muestreo o realizar aplicaciones fitosanitarias. En este mismo estudio se observó que los frutos ocupados por ninfas de *P. kellyanus* tienden a caer en mayor proporción que los que no fueron ocupados por ninfas.

El siguiente objetivo de esta tesis fue determinar la eficacia de tres insecticidas (clorpirifos, spinosad y spirotetramat) en el control de las ninfas de *P. kellyanus*. Se seleccionaron estos insecticidas por tener un modo de acción diferente. Clorpirifos y spinosad mostraron una elevada eficacia contra las ninfas de *P.*

kellyanus y redujeron significativamente el porcentaje de frutos dañados cuando se dió una sola generación de ninfas. Sin embargo, su persistencia no fue suficiente para evitar el ataque de una segunda generación de ninfas de *P. kellyanus*. Spirotetramat no presentó un efecto de choque contra esta plaga y tampoco evitó el ataque de una segunda generación. Por último, en estos trabajos de campo, se analizaron los efectos secundarios de estos tres insecticidas sobre los enemigos naturales presentes en el momento de los tratamientos. Tanto spinosad como spirotetramat afectaron negativamente a las poblaciones fitoseidos en campo. Debido a las bajas poblaciones del resto de enemigos naturales no se pudo evaluar los efectos secundarios sobre estos. Por ello, se decidió estudiar los efectos secundarios de estos insecticidas sobre coccinélidos y parasitoides en condiciones de laboratorio.

En primer lugar se determinaron los efectos letales y subletales de spirotetramat (ya se conocían los efectos secundarios de clorpirifos y spinosad en coccinélidos) en adultos y larvas de *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) en condiciones de laboratorio, por aplicación directa de los productos y por ingestión de presa, *Planococcus citri* Risso (Hemiptera: Pseudococcidae), previamente tratada por los productos. Spirotetramat resultó inocuo cuando se aplicó directamente sobre larvas o adultos de *C. montrouzieri*, y además no afectó a la supervivencia, longevidad fecundidad, fertilidad y y supervivencia de la descendencia. Cuando adultos y larvas de *C. montrouzieri* fueron alimentados con presa previamente tratada, spirotetramat también resultó ser inocuo.

En el siguiente estudio se determinaron los efectos letales y subletales de spirotetramat, spinosad y clorpirifos sobre el parasitoide *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), principal parasitoide del piojo rojo de California *Aonidiella aurantii* (Maskell) (Hemipteta: Diaspididae) en cítricos. En adultos, tanto clorpirifos como spinosad fueron clasificados como tóxicos mientras que spirotetramat resultó moderadamente tóxico. Sobre inmaduros de *A. melinus*, clorpirifos resultó moderadamente tóxico, spirotetramat ligeramente tóxico y spinosad inocuo. Teniendo en cuenta estos resultados, spirotetramat se podría utilizar contra *P. kellyanus* en parcelas con niveles poblacionales bajos cuando haya que tratar también contra *A. aurantii* puesto que presenta una alta eficacia contra esta plaga.

Resum

Pezothrips kellyanus (Bagnall) (Thysanoptera: Thripidae) és una plaga de cítrics al Mediterráni descrita per primera vegada a Austràlia al segle passat. El 2007 es van observar els primers danys als nostres cítrics, concretament a Alzira, a la comarca de la Ribera Alta. Actualment, és una plaga més dels cítrics valencians. Les nimfes de *P. kellyanus* es refugien i s'alimenten de la superfície dels fruits recentment quallats, això produeix unes escarificacions característiques circulars al voltant del peduncle. Els danys són només estètics i devaluen el seu valor al mercat, podent arribar al 100% de fruits danyats.

Hui en dia, el control químic és pràcticament l'única alternativa contra aquest trips, els danys són molt variables en funció de la varietat, la zona i també els anys. Per poder millorar la gestió integrada de *P. kellyanus* és necessari un major coneixement sobre la seua biologia i ecologia en camp. Per a això, s’ha avaluat la dinàmica poblacional de les nimfes de *P. kellyanus* durant el període on els fruits recentment quallats són més sensibles als danys produïts per les nimfes. A més, s'ha estudiat la distribució de la plaga dins dels arbres i el seu moviment al llarg del dia, factors necessaris tant per a estimar la població de trips com per afinar les aplicacions de productes fitosanitaris. Tant la dinàmica com els danys van variar segons parcel·les i anys, després de la caiguda de pètals es van observar una o dues generacions de nimfes que van produir danys als fruits. Aquest resultat subratlla la necessitat de realitzar mostrejos setmanals des de la caiguda de pètals i continuar, fins i tot, després del tractament contra la primera generació. A més, segons les dades obtingudes, quan es dóna una segona generació de *P. kellyanus*, aquesta és més perjudicial per als fruits.

Les nimfes de *P. kellyanus* van mostrar un clara preferència pels fruits situats a la part alta de la copa, el que va coincidir amb el major nombre de fruits danyats en aquesta zona. No obstant això, la distribució de les nimfes va ser uniforme en les quatre orientacions de l'arbre i la seva abundància tampoc va variar al llarg del dia. Per tant, l'orientació i el moment del dia no semblen ser claus a l'hora de desenvolupar un pla de mostreig o realitzar aplicacions fitosanitàries. En aquest mateix estudi es va observar que els fruits ocupats per nimfes de *P. kellyanus* tendeixen a caure en major proporció que els que no van ser ocupats per nimfes.

El següent objectiu d'aquesta tesi va ser determinar l'eficàcia de tres insecticides (clorpirifos, spinosad i spirotetramat) en el control de les nimfes de *P. kellyanus*. Es van seleccionar aquests insecticides per tenir una manera d'acció diferent. Clorpirifos i spinosad van mostrar una elevada eficàcia contra les nimfes de *P. kellyanus* i van reduir significativament el percentatge de fruits danyats quan es va donar una sola generació de nimfes. No obstant això, la seva persistència no va ser

suficient per evitar l'atac d'una segona generació de nimfes de *P. kellyanus*. Spirotetramat no va presentar un efecte de xoc contra aquesta plaga i tampoc va evitar l'atac d'una segona generació. Finalment, en aquests treballs de camp, s'ha analitzar els efectes secundaris d'aquests tres insecticides sobre els enemics naturals presents en el moment dels tractaments. Tant spinosad com spirotetramat van afectar negativament les poblacions fitoseids en camp. A causa de les baixes poblacions de la resta d'enemics naturals no es va poder avaluar els efectes secundaris sobre ells. Per això, es va decidir estudiar els efectes secundaris d'aquests insecticides sobre coccinèlids i parasitoids en condicions de laboratori.

En primer lloc es van determinar els efectes letals i subletals de spirotetramat (ja eren coneguts els efectes secundaris de clorpirifos i spinosad en coccinèl·lids) en adults i larves de *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) en condicions de laboratori, per aplicació directa dels productes i per ingestió de presa, *Planococcus citri* Risso (Hemiptera: Pseudococcidae), prèviament tractada pels productes. Spirotetramat va resultar innocu quan es va aplicar directament sobre larves o adults de *C. montrouzieri*, a més no va afectar la supervivència, longevitat fecunditat, fertilitat ii supervivència de la descendència. Quan adults i larves de *C. montrouzieri* van ser alimentats amb presa prèviament tractada, spirotetramat també va resultar ser innocu.

El següent estudi es van determinar els efectes letals i subletals de spirotetramat, spinosad i clorpirifos sobre el parasitoide *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), principal parasitoid del poll roig de Califòrnia *Aonidiella aurantii* (Maskell) (Hemipteta: Diaspididae) en cítrics. En adults, tant clorpirifos com spinosad van ser classificats com a tòxics mentre que spirotetramat va resultar moderadament tòxic. Sobre immadurs de *A melinus*, clorpirifos va resultar moderadament tòxic, spirotetramat lleugerament tòxic i spinosad innocu. Tenint en compte aquests resultats, spirotetramat es podria utilitzar contra *P. kellyanus* en parcel·les amb nivells poblacionals baixos quan calgui tractar també contra *A. aurantii* ja que presenta una alta eficàcia contra aquesta plaga.

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Introducción

1.1 Cultivo de los cítricos

1.1.1 Importancia económica

El sector citrícola es uno de los más importantes de la agricultura española. España, con una producción anual de unos 5,5 millones de toneladas de cítricos en 2013 (MAGRAMA, 2014), principalmente naranjas, mandarinas y limones, es el sexto productor mundial y el primero para el mercado fresco (FAOSTAT 2013). La comercialización de la fruta fresca exige una gran calidad estética y cualquier lesión puede depreciar su valor. Por otro lado, el hecho de que aproximadamente la mitad de la fruta producida sea destinada para exportación requiere, también, la ausencia absoluta de organismos que estén en los listados de cuarentena de los países a los que se exporta. Ambos condicionantes exigen un nivel de conocimiento muy elevado de las diferentes plagas y enfermedades que les afectan.

Por otra parte, el 1 de enero de 2014 entró en vigor la directiva europea 2009/128/CE que tiene por objetivo conseguir un uso sostenible de los plaguicidas en los países miembros con objeto de reducir sus riesgos y sus efectos en la salud humana y en el medio ambiente. Dicha directiva, propone, entre otros aspectos, que la aplicación de los principios generales de la Gestión Integrada de Plagas (GIP) sea obligatoria para todos los productores europeos, promoviendo el desarrollo y la implementación de la GIP que aboga por la búsqueda de nuevos métodos de control alternativos, tanto biológicos como biotecnológicos (EU 2009). Por lo tanto, la alta exigencia de fruta libre de daños e insectos de cuarentena y la nueva normativa europea marcan el presente y futuro de la gestión de plagas en nuestros cítricos.

1.1.2 Gestión Integrada de Plagas en cítricos

La gestión integrada de plagas (GIP) es una estrategia de control que consiste básicamente en la aplicación racional de una combinación de medidas biológicas, biotecnológicas, químicas, de cultivo o de selección de vegetales, de modo que la utilización de productos fitosanitarios se limite al mínimo necesario (Naranjo et al. 2015). En la actualidad se considera que la lucha integrada o el manejo integrado de plagas es el único sistema racional y capaz de dar soluciones a largo plazo y de forma sostenible a los problemas de plagas.

En cítricos, como en otros cultivos, la aplicación correcta de un programa GIP requiere, entre otros aspectos, el conocimiento completo sobre la biología de las especies plaga y sus enemigos naturales, los métodos de muestreos que permitirá establecer los umbrales de tratamiento, la manipulación del agroecosistema para mantener la población de enemigos naturales, el conocimiento sobre la eficacia de los plaguicidas y sus efectos secundarios sobre la fauna auxiliar (Urbaneja et al. 2015). La dificultad de su integración en cítricos estriba en el uso de plaguicidas de síntesis. Tradicionalmente, el control de plagas en cítricos se ha basado en la aplicación de estos plaguicidas. El uso indiscriminado de estos productos afecta negativamente a los enemigos naturales, que en condiciones normales controlan otros fitófagos con potencial para causar daños económicos en este cultivo (plagas ocasionales y secundarias), complicando así la integración de la GIP (Trumper y Holt 1998; Naranjo et al. 2015).

La GIP en cítricos se aplica en varios países productores. En California (EEUU), el piojo rojo de California *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) y *Coccus pseudomagnoliarum* Kuwana son las principales plagas que han condicionado los programas de GIP hasta la reciente entrada de *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Grafton-Cardwell et al. 2013). *Aonidiella aurantii* fue la principal plaga hasta mediados de 1980, cuando comenzaron las sueltas masivas del parasitoide *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) (Figura 1.1) y el uso de plaguicidas selectivos para este parasitoide (Moreno y Luck 1992; Vasquez y Morse 2012).

Figura 1.1. Adulto de *Aphytis melinus* sobre fruto infestado de *Aonidiella aurantii*.

En Florida, la mayoría de la producción de cítricos se destinaba para zumo y recibía muy pocos tratamientos con insecticidas de amplio espectro, permitiendo la acción de los enemigos naturales. No obstante, la llegada del minador de los cítricos *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) y sobre todo de *Diaphorina citri* Kuwayama (Homoptera:Psyllidae) (Figura 1.2), vector de la bacteria '*Candidatus* Liberibacter asiaticus' a la que se le atribuye ser el agente causal de la devastadora enfermedad "Huanglongbing" (HLB) o "greening" de los cítricos (Grafton-Cardwell et al. 2013), ha cambiado radicalmente la GIP. Actualmente se están realizando de nuevo tratamientos continuados con insecticidas de amplio espectro (Michaud 2004; Qureshi et al. 2009). Sin embargo, incluso bajo estas condiciones de alta presión, se está recomendando estrategias de gestión en base a umbrales de tratamiento porque son más rentables que los tratamientos a calendario programado (Monzo and Stansly 2015).

Figura1.2 . Adulto de *Diaphorina citri* Kuwayama vector del 'huanglongbing' (HLB) (Monzó et al. 2015).

En Australia, *A. aurantii* es considerada también la principal plaga y su control está gestionado mediante la sueltas masivas del parasitoide *A. melinus* dentro de los programas de GIP (Smith et al. 1997; Papacek 2006).

En los cítricos españoles, la mayor parte de las plagas potenciales están reguladas por sus enemigos naturales (depredadores, parasitoides y entomopatógenos), tanto autóctonos como naturalizados, que consiguen un excelente control biológico y facilitan la adopción de programas de GIP (Jacas y Urbaneja 2010). Por ejemplo, el ácaro rojo

Panonychus citri (McGregor) (Acari: Tetranychidae) está controlado por el fitoseido *Euseius stipulatos* (Athias-Henriot) (Acari: Phytoseiidae) de forma natural en naranjos. Por otra parte, la introducción del coccinélido *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinelidae) contra la cochinilla acanalada *Icerya purchasi* Maskel (Hemiptera:Margarodidae), el parasitoide *Cales noacki* Howard (Hymenoptera: Aphelinidae) contra la mosca algodonosa *Aleurothrixus floccosus* (Maskell) (Hemiptera:Aleyrodidae) o *Aphytis lepidosaphes* Compere (Hymenoptera: Aphelinidae) contra la serpeta gruesa *Lepidosaphes beckii* (Hemiptera: Diaspididae) son algunos ejemplos de control biológico clásico en nuestros cítricos (Jacas y Urbaneja 2010).

Sin embargo, la mayoría de la producción de cítricos se destina al consumo en fresco lo que repercute en aquellas plagas que afectan directamente al fruto por afectar a su estética. Estas plagas están sujetas a umbrales de tratamientos muy bajos, y en muchas ocasiones, el control biológico de algunos fitófagos no es adecuado y sus densidades poblacionales sobrepasan el umbral económico de daños, llegando a considerarse plagas clave en el cultivo de los cítricos. Este es el caso del diaspídido *A. aurantii* que tiene preferencia por fijarse en el fruto y es considerada una plaga clave en el agrosistema de cítricos español y, por lo general, se necesitan aplicaciones de productos químicos con el fin de mantener las infestaciones por debajo de los umbrales económicos (Pekas 2010). Para mejorar su gestión se ha desarrollado la técnica de confusión sexual de machos de *A. aurantii, que* es totalmente respetuosa y compatible con el control biológico de esta plaga, e incluso puede tener efectos beneficiosos sobre los enemigos naturales (Vanaclocha 2012). Además se están poniendo a punto sueltas masivas del parasitoide *A. melinus* que han resultado exitosas en otros países (Moreno and Luck 1992)**.**

Al igual que ocurre con las plagas que afectan a los frutos, las plagas de cuarentena tienen umbrales de tratamientos muy bajos. En España en general, y en la Comunidad Valenciana en particular, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) representa una plaga clave del cultivo de los cítricos (Beitia et al. 2003). Por su status de cuarentena en muchas zonas, la fruta que se exporta debe estar libre de mosca. Actualmente la protección fitosanitaria contra esta plaga se enfoca hacia la Gestión Integrada o Manejo Integrado de Plagas (GIP o MIP). En la Comunidad Valenciana, en el marco de esta GIP en cítricos, se ha apostado por la implantación de la Técnica del Insecto Estéril (TIE) como sistema básico para la reducción de poblaciones de la mosca (Primo-Millo et al. 2003).

Además de estas dos plagas principales, los clementinos, por sus características fisiológicas, son atacados por otras dos plagas: los pulgones *Aphis spiraecola* Patch y *A. gossypii* Glover (Hemiptera: Aphididae) y la araña roja *Tetranychus urticae* Koch (Acari: Tetranychidae) (Tena y García-Marí 2011). Actualmente se está trabajando para mejorar el control de los pulgones mediante el uso de cubiertas vegetales que favorezcan la presencia de otras especies de pulgones que no sean plagas de cítricos y, a la vez, permitan incrementar el número de depredadores generalistas antes de la llegada de *A. spiraecola* y *A. gossypii* (Gomez-Marco et al. 2015, 2016 , 2016 en prensa).

Tetranychus urticae es uno de los ácaros tetraníquidos más perjudiciales (Jacas y Urbaneja 2008; Abad-Moyano et al. 2008; Jacas y Urbaneja 2010; Abad-Moyano et al. 2010a). Se ha demostrado que el uso de cubiertas vegetales con la gramínea *Festuca arundinacea* (Poaceae) mejora su gestión (Aguilar-Fenollosa et al. 2011a; Aguilar-

Fenollosa et al. 2011b; Aguilar-Fenollosa et al. 2011c). Los ácaros depredadores *Neoseiulus californicus* (McGregor) y, especialmente, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) se consideran especies clave para el control biológico de *T. urticae* en cítricos en España (Argolo et al. 2014). El éxito de las sueltas de ambos depredadores se ha demostrado en ensayos de semi-campo con clementinos (Abad-Moyano et al. 2010), aunque no se ha podido demostrar la eficacia de sus sueltas masivas en campo.

1.1.3 Jerarquización de fitófagos plaga

En comparación con otros cultivos agrícolas, los cítricos se caracterizan por ser un ecosistema rico tanto en fitófagos como en enemigos naturales. En este agroecosistema los fitófagos más importantes pueden llegar a agruparse según el nivel de control que ejerzan sus enemigos naturales. De esta manera, y simplificando esta agrupación nos podemos encontrar en la actualidad con plagas perfectamente controladas por sus enemigos naturales (cuyas oscilaciones poblacionales se encuentran muy por debajo de sus umbrales económicos de daños (UED) (Ejem. *Icerya purchasi* e *Insulaspis gloverii*), otras cuyo control es bastante satisfactorio (con oscilaciones poblacionales que sólo en ocasiones superan los UED: *A. floccosus*, *P. citri*, *Chrysomphalus dyctiospermi* (Morgan) (Hemiptera: Diaspididae), *Coccus hesperidium* Lineo, *Ceroplastes sinensis* Del Guercio, *Planococcus citri* y *Saissetia oleae* (Olivier) (Hemiptera:Coccidae)) y otras como las comentadas en el punto anterior mal controladas (cuyas oscilaciones poblacionales, de forma natural, superarían todos los años los UED. La clave del éxito la GIP en cítricos es mantener a los fitófagos incluidos en este último grupo por debajo de sus respectivos UED sin perturbar el control natural del resto de fitófagos. Para ello, en la actualidad sigue siendo necesario realizar tratamientos químicos para evitar los daños de las plagas mal controladas de forma natural, siendo la elección de estos productos fitosanitarios una de las bases del GIP en cítricos. Estos plaguicidas deben de ser seleccionados además de por su alta eficacia contra las plagas a los que van dirigidos y de un buen perfil ecotoxicológico, por ser selectivos para los enemigos naturales que controlan a los dos primeros grupos de plagas, de manera que no pongan en peligro el control biológico de éstos (Jacas y Urbaneja 2010).

1.1.3 Plagas invasoras

Actualmente, la globalización ha facilitado el transporte de personas y materiales por todo el mundo en proporciones y a un ritmo sin precedentes y, con ellas, lo hacen también un gran número de organismos. De este modo, se ha observado un aumento exponencial del número de especies exóticas e invasoras en las últimas décadas (Roques et al. 2009). En un primer momento los insectos invasores carecen de enemigos naturales capaces de limitar su expansión y, si el ambiente es propicio, pueden llegar a aumentar sus niveles de poblacionales hasta alcanzar el nivel de plagas clave y desbaratar los programas de GIP establecidos antes de su llegada y aclimatación. El caso de *D. citri* en Florida y la posterior detección de HLB, comentado anteriormente, es un claro ejemplo de cómo la llegada de una nueva plaga puede afectar a los programas de GIP en cítricos.

Los cítricos españoles no son una excepción a esta tendencia y en las últimas décadas son numerosas las introducciones de nuevas plagas. Aproximadamente, se estima que cada 4-5 años llega y se establece una nueva plaga. Dos de los ejemplos más conocidos por su trascendencia han sido la mosca blanca algodonosa *A. floccosus* en los años setenta y el minador *P. citrella* en los noventa. Más recientemente, se han detectado el cóccido *C. pseudomagnoliarum* (Tena y Garcia-Marí 2008), el trips *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) (Navarro et al. 2008a), el cotonet de les Valls *Delottococcus aberiae* (De Lotto) (Hemiptera: Pseudococcidae) (Beltrà et al. 2013) o el psílido *Trioza erytreae* (Pérez-Otero, 2015).

Por su pequeño tamaño y comportamiento críptico, los trips están considerados como uno de los principales grupos de insectos invasores (Morse y Hoddle 2006). La mayoría de especies de trips invasoras presentan una alta fecundidad y generaciones cortas, generalmente con predisposición a la partenogénesis, evitando así la necesidad de cópulas. En algunos casos las combinaciones de estas características los hacen también propensos al desarrollo de resistencias a insecticidas (Kirk y Terry 2003; Lewis 1997; Mound et al. 2001; Mound y Telon 1995; Shelton et al. 2003; Worner 2002; Espinosa et al. 2002; Bielza 2008; Bielza et al. 2007). En este contexto se sitúa el trips *P. kellyanus*, un trips originario probablemente de Oceanía que a partir de finales del siglo XX empezó a producir daños estéticos en los cítricos e invadir nuevas áreas citrícolas, convirtiéndose en una plaga más de los cultivos de cítricos.

1.1.4 Los trips como plagas de cítricos

Un gran número de especies de trips están relacionadas con el agrosistema de los cítricos, sin embargo, pocas especies pueden considerarse plagas en las diferentes áreas citrícolas (Lewis, 1997). Entre ellas podemos encontrar: *Frankliniella bispinosa* (Morgan), *Frankliniella occidentalis* (Pergande), *Heliothrips haemorrhoidalis* (Bouche), *P.*

kellyanus (Bagnall), *Scirtothrips aurantii* Faure, *S. citri* (Moulton), *S. dorsalis* Hood, *S. inermis* Priesner y *Thrips major* Uzel, *T. meridionalis* (Priesner) o *T. tabaci* Lindeman, (Ebeling 1959; Lacasa y Llorens 1998; Blank y Gill 1997; Parker y Skinner 1997; Bedford 1998). La mayoría de estas especies son polífagas y se encuentran en las flores sin llegar a causar graves daños económicos (Lacasa et al. 1996; Navarro et al. 2008b).

En general, los daños producidos por los trips en cítricos son de tipo estético. Las ninfas se alimentan de los frutos recién cuajados (Figura 1.3) produciendo escarificaciones circulares alrededor del pedúnculo que varían de tamaño en función de la especie de trips y la variedad atacada (Navarro et al. 2008b).

Figura 1.3. Ninfas de *P. kellyanus* alrededor del pedúnculo de un fruto recién cuajado.

Se han descrito otros tipos de daños, como son el plateado de los frutos maduros o la quemadura de las hojas entre otros (Lacasa et al. 1996;

Navarro et al. 2008ab). En la cuenca mediterránea, pueden encontrarse tres especies de trips que pueden causar daños importantes. Los frutos maduros pueden ser atacados ocasionalmente por *H. haemorrhoidalis* (Longo 1986; Lacasa et al. 1996). *Scirtothrips inermis*, puede producir daños localizados en diferentes zonas de Alicante, Castellón, Murcia y Valencia (Lacasa et al. 1996; EPPO 2005). Por último, *P. kellyanus,* se ha convertido en una seria plaga de cítricos, como se ha comentado anteriormente (Marullo 1998; EPPO 2006; Vassiliou 2007; Varikou 2009).

1.2 *Pezothrips kellyanus* como plaga de cítricos

1.2.1 Origen y distribución geográfica

Pezothrips kellyanus, también conocido como Pezothrips en castellano o Kelly's citrus thrips en inglés, fue descrita inicialmente en Australia como *Physothrips kellyanus* (Bagnall 1916), posteriormente se citó como *Taeniothrips* (Physothrips) *kellyanus* (Webster et al. 2006) y más tarde se transfirió al género *Megalurothrips* Bagnall (Bhatti 1969). Finalmente, se incluyó en el género *Pezothrips* Karny (zur Strassen 1996) junto a ocho especies originarias del sur paleártico (Mound y Gillespie 1997). La similar morfología con las especies incluidas en el género *Pezothrips* sugería que *P. kellyanus* era originaria de la misma parte del mundo. De hecho, desde 1997 hasta el año 2006 se consideraba que la especie no era originaria sino introducida en Australia y debía ser considerada nativa del área mediterránea. Sin embargo, posteriormente se encontró en plantas nativas de Australia, lo que sugiere que es un insecto nativo de Australia. Lo que implica que haya invadido el sur de Europa en los últimos años del siglo XX, donde ha cambiado sus hábitos y plantas de las que se alimenta (Webster et al. 2006; Reynaud 2010; Navarro-Campos et al. 2013).

Hasta la primera mitad del siglo XX se encontraba exclusivamente en Australia. En 1950 se capturó por vez primera fuera de Australia, en Nueva Zelanda (Mound y Walker 1982). Zur Strassen (1986) cita la especie por vez primera en el Mediterráneo en Grecia y posteriormente zur Strassen (1996, 2003) la describe también en Turquía, España y Creta. En 1998 se cita en Italia (Marullo 1998) y en 2004 en Chipre y el sur de Francia (Moritz et al. 2004). Costa et al. (2006) descubren que en 2002 y 2003 la especie era muy abundante en limoneros de Portugal. Respecto al continente americano, se ha detectado ya en dos países, Hawaii (Hawaii Department of Agriculture 2006) y Chile (EPPO 2012). Recientemente se han observado los daños y la presencia de *P. kellyanus* en cítricos de Túnez (Bellam y Boulahia-Kheder 2012), por lo que también se encuetra en África.

A pesar de haberse detectado su presencia en los años ochenta, los primeros daños en la zona del Mediterráneo no se describen hasta finales de los noventa en Sicilia (Marullo 1998) y Creta (Varikou et al. 2002). En nuestros cítricos, se observaron los daños por primera vez en el año 2007, cuando apareció un foco en parcelas de naranjo Valencia Late situadas en Alzira, en la comarca de la Ribera Alta. Posteriormente se pudo comprobar, en un conteo realizado en trampas pegajosas que el insecto ya estaba en parcelas de la comarca del Baix Segura en el año 2005 (Navarro et al. 2008b). Actualmente, *P. kellyanus* se encuentra distribuido por toda la Comunidad Valenciana y produce daños principalmente en la comarca de La Ribera-Alta y La Safor, si bien las zonas afectadas son cada vez mayores.

1.2.2 Descripción morfológica

Los adultos de *P. kellyanus* son fácilmente visibles en las flores de los cítricos donde se reconocen por presentar una coloración oscura, variando del marrón al negro (Figura 1.4). Los estadios inmaduros presentan una coloración que va del blanco al naranja oscuro y son más difíciles de observar debido a su comportamiento tigmotáxico que les lleva a situarse en zonas muy protegidas como la unión del fruto con el cáliz (Purvis 2002; Baker 2006; Webster et al. 2006).

Figura 1.4 . Adultos de *P. kellyanus* en flor de cítricos.

Los adultos de *P. kellyanus* tienen un tamaño que varía entre 1,6 y 1,8 mm aproximadamente en el caso de las hembras (en algunos casos puede llegar a 2 mm) y de 1,2-1,6 mm aproximadamente en el caso de los machos (Bagnall 1916).

Las hembras tienen el cuerpo de color marrón oscuro, con los tarsos de color amarillo y los segmentos antenales III y IV en la zona apical de color claro o amarillo (Figura 1. 5). Las alas anteriores son de color marrón y en su base más claras (Figura 1.6). Las antenas están formadas por ocho segmentos oscuros exceptuando las zonas de unión de los artejos 3 y 4 que son transparentes (Bagnall 1916) (Figura 1. 7). La cabeza es un poco más ancha que larga y tiene tres pares de setas ocelares, siendo el par situado entre los ocelos extremadamente largo (Figura 1.8).

Figura 1. 5. Hembra adulta de *P. kellyanus* PADIL 2014; Hoddle et al. 2014).

Figura 1. 6. Alas de adulto de *P. kellyanus* (PADIL 2014; Hoddle et al. 2014).

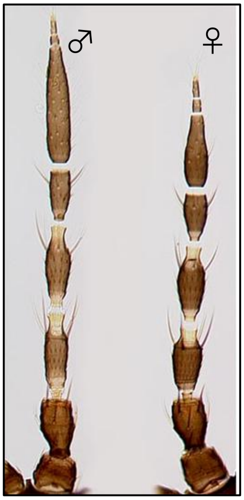


Figura 1. 7. Antena de macho y hembra de *P. kellyanus* (PADIL 2014; Hoddle et al. 2014).

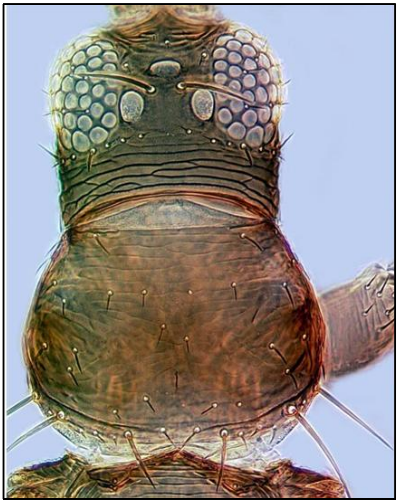


Figura 1. 8. *Cabeza y pronoto de P. kellyanus* (PADIL 2014; Hoddle et al. 2014).

El octavo segmento abdominal de las hembras tiene varias características útiles para reconocer la especie. Una de ellas es la ausencia de ctenidias (líneas de microsetas) en los laterales del segmento, presentando en su lugar grupos irregulares de quetas. Otra característica es la presencia de microsetas en los laterales del borde inferior de este mismo segmento, faltando en la parte central (Figura 1.9 y Figura 1. 10) (Mound y Walker 1982; Marullo 1998; Moritz et al. 2004; Webster et al. 2006).

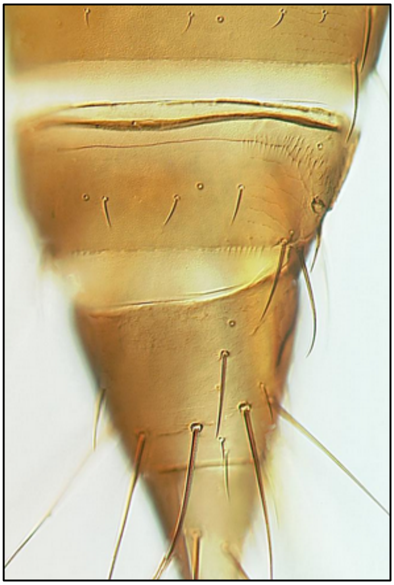


Figura 1.9. Térgitos VII-X (PADIL 2014; Hoddle et al. 2014).

Figura 1. 10. Térgito abdominal VIII (PADIL 2014; Hoddle et al. 2014).

Los machos son similares a las hembras pero más pequeños y estrechos (Figura 1.11). Además, presentan el sexto artejo antenal inusualmente largo, siendo aproximadamente el doble de largo que en la hembra (Figura 1.7).

Igualmente, los machos de esta especie se distinguen por presentar en los segmentos abdominales más de 25 pequeños poros circulares (Figura 1.12) (Hoddle et al. 2014; PADIL 2014).

Figura 1.11. Macho adulto *de P. kellyanus* (PADIL 2014; Hoddle et al. 2014).

Figura 1.12. Esternitos abdominales de macho adulto de *P. kellyanus* (PADIL 2014; Hoddle et al. 2014).

Los trips son insectos heterometábolos y por lo tanto la denominación de los estadios inmaduros correcta es la de ninfas. Sin embargo, algunos autores les han denominado como larvas y pupas porque los trips tienen un tipo de desarrollo intermedio entre los holometábolos y heterometábolos (Vance 1974; Moritz 1997; Milne et al. 1997; Chapman 1998; Morse y Hoddle 2006; Mound 2007; Vierbergen et al. 2010; Navarro-Campos 2013).

Las ninfas del primer estadio son blanquecinas o amarillentas (Figura 1.13) y las de segundo se tornan anaranjadas al final de su desarrollo (Figura 1.14). Estas últimas presentan unos dientes esclerotizados en el octavo segmento abdominal que son útiles para su identificación y que presumiblemente les facilitan el movimiento entre las partículas del suelo cuando van a pupar (Kirk 1987; Webster et al. 2006; Navarro et al. 2009).

Figura 1.13. Ninfa de primer estadío de *P.* kellyanus.

Figura 1.14. Ninfa de segundo estadío de *P. kellyanus*.

1.2.4 Ciclo biológico

Las hembras de *P. kellyanus* ponen sus huevos en las partes tiernas de la planta, sobre todo en los pétalos de las flores de cítricos (Baker 2006). Como todos los trips del suborden Terebrantia*,* tienen dos estadios ninfales y dos estadios inactivos en los que no se alimentan (prepupa y pupa) (Lewis 1997). En cítricos, las ninfas se establecen dentro de las flores y sobre los frutos recién cuajados o en proceso de crecimiento, generalmente en zonas muy protegidas: en la zona de unión del fruto y el cáliz, entre frutos en contacto. Las ninfas de segundo estadio se dejan caer al suelo para pupar. Los adultos al emerger se dirigen a la parte aérea para alimentarse y reproducirse. Los machos suelen formar agrupaciones conocidas en inglés como “leks” en hojas terminales jóvenes y las hembras son atraídas a dichas agregaciones para aparearse (Baker et al. 2002; Baker 2006; Mound y Jackman 1998)(Figura 1.15).

Figura 1.15. Agrupación de adultos de *P. kellyanus* en envés de hojas próximas pequeños frutos.

En condiciones de laboratorio se ha estimado que el desarrollo completo desde huevo a adulto tarda 10 días a 32,5°C y 40 días a 15°C, siendo los umbrales de temperatura estimados de 10,2 y 33°C (Varikou et al. 2009).

1.2.5 Abundancia estacional

El número de generaciones por año en cítricos varía según autores. Así, Vassiliou (2007) afirma que puede desarrollar más de seis generaciones al año, mientras que Blank y Gill (1997) consideran que tiene una generación en la mayoría de variedades de cítricos que florecen en primavera. Se sabe que las mayores abundancias poblacionales de *P. kellyanus* en campos de cítricos coinciden con la floración primaveral y el principal periodo de realización del daño ocurre en las 6-8 semanas que siguen a la caída de pétalos pero no se conoce con exactitud la evolución estacional a lo largo del año de la abundancia del trips en parcelas de cítricos (Navarro-Campos et al. 2012a). Además, existe cierta controversia respecto al número de generaciones que se desarrollan al año en cítricos y, lo que es más importante para su gestión, aquellas que causan daños. Conocer el número de generaciones que dañan los frutos permitirá determinar el número de tratamientos necesarios para disminuir los daños producidos y facilitará la elección de los insecticidas.

1.2.6 Daños producidos

Los principales daños producidos por *P. kellyanus* se deben a la alimentación de las ninfas de los frutos recién cuajados. Al alimentarse destruyen la pigmentación verde de las células epidérmicas produciendo manchas decoloradas irregulares (Blank y Gill 1997). Estas manchas dan lugar a una escarificación o cicatriz alrededor del pedúnculo cuando el fruto crece (Figura 1.16). La escarificación circular puede ser parcial o completa y va alejándose del pedúnculo a medida que el fruto crece. Puede ir acompañada de otras lesiones situadas en la parte lateral o en la base del fruto. Estas lesiones se supone que se producen por la presencia de pétalos que se han quedado pegados al fruto o a frutos que están en contacto y sirven de refugio a las ninfas.

Figura 1.16. Daños observados en frutos maduros producidos por *P. kellyanus*

Por otra parte, los adultos pueden producir lesiones sobre frutos maduros cuando se alimenta de estos. Los frutos aparecen con zonas plateadas o decoloradas, normalmente en las zonas en contacto de frutos entre sí, o entre frutos y hojas (Blank y Gill 1997; Webster et al. 2006; Vassiliou 2007). El daño en frutos maduros es menos común pero más severo pudiendo llegar a cubrir el fruto enteramente (Vassiliou 2007).

El porcentaje de frutos dañados es muy variable según variedades, características de las parcelas y condiciones ambientales (Navarro-Campos et al. 2013). Además, los daños producidos por *P. kellyanus* se pueden confundirse con las lesiones producidas por microlepidópteros como *Anatrachyntis badia* (Hodges) (Lepidoptera Cosmopterigidae) o por el roce inducido por el viento (Navarro-Campos et al. 2010). En general, se considera que *P. kellyanus* está presente en todas las variedades pero produce daños principalmente en narajas del grupo Navel, pomelos y limones (Baker et al. 2002; Blank and Gill 1997; Conti et al. 2001, 2004; Varikou et al. 2002; Vassiliou 2007,2011).

1.3 Gestión integrada de *Pezothrips kellyanus*

De los tres grupos de cítricos afectados en mayor medida por *P. kellyanus*, el grupo Navel es el más abundante en nuestros cítricos y por tanto donde más daños se han observado. Hasta la llegada de *P. kellyanus,* y más recientemente la del cotonet de les Valls *D. aberiae,* sólo *A. aurantii* causaba daños en las naranjas del grupo Navel y era necesario realizar tratamientos con insecticidas. Como se ha comentado anteriormente, actualmente se han desarrollado varias estrategias de control, como son la suelta masiva del parasitoide *A. melinus* y la confusión sexual, que permitían controlar este diaspídido sin realizar tratamientos. Por lo tanto, la llegada y establecimiento de *P. kellyanus* ha alterado la GIP en las naranjas del grupo Navel puesto que actualmente se realizan entre uno y tres tratamientos tras la caída de pétalos (Conti et al. 2004; Vassilou 2007; 2011; Navarro-Campos et al. 2012a).

En cuanto a los umbrales de tratamiento, Navarro-Campos et al. (2012a) recomiendan realizar tratamientos cuando el más del 12% de los frutos recién cuajados están ocupados por ninfas de *P. kellyanus*. Además, varios documentos técnicos sugieren muestrear 100 frutos y tomar como umbral de tratamiento un 5 a 10% de frutos ocupados por ninfas (Perrotta et al. 2004; Baker 2006; Jackman et al. 2011; Urbaneja et al. 2015).

Aunque se han realizado varios ensayos sobre el efecto de diferentes insecticidas químicos para el control del trips, los organofosforados son los insecticidas más utilizados. De hecho, debido al reiterado uso de clorpirifos contra *P. kellyanus* en Australia ya se han encontrado poblaciones resistentes (Purvis 2003; Baker 2006). Para mejorar su gestión e impedir la aparición de resistencias es necesario no solo determinar la eficacia de los insecticias para disminuir los daños como se ha hecho hasta ahora (Conti et al. 2001; Vassiliou 2007), sino también establecer si afectan a los enemigos naturales y por tanto alteran el actual control biológico natural establecido en nuestros cítricos y en especial en las variedades del grupo Navel. Además, se debería determinar si un segundo tratamiento es necesario en función del número de generaciones que atacan a los frutos y la eficacia y persistencia de los insecticidas utilizados. Por último, se desconoce la distribución de las ninfas en la copa de los árboles en el momento que producen los daños. Esta información facilitaría el muestreo y permitiría focalizar la aplicación de los insecticidas en los árboles.

En resumen, el control químico es todavía la única herramienta disponible en el mercado para combatir a esta nueva plaga de cítricos y hasta que se desarrolle una herramienta más respetuosa (Navarro-Campos et al. 2012a) es necesario mejorar y ampliar el conocimiento del control químico para no alterar, o alterar lo menos posible, el control biológico existente en nuestros cítricos. Dentro de este gran objetivo se enmarca la presente tesis doctoral.

CAPÍTULO 2

Justificación y Objetivos

Pezothrips kellyanus es una de las últimas plagas que ha llegado a nuestros cítricos. Hoy en día el control químico es prácticamente la única alternativa contra este trips, cuyos daños son muy variables en función de la variedad, la zona y también de los años. Tanto para estimar la población de trips como para afinar las aplicaciones de productos fitosanitarios, el **primer objetivo** de esta tesis fue **esclarecer el número de generaciones que pueden atacar a los frutos recién cuajados** ya que se dieron dos generaciones de ninfas durante el periodo en el que el fruto es más susceptible a su ataque. Además, **se evaluó si existían diferencias en los daños ocasionados por las ninfas de la primera y segunda generación** que atacó a los frutos.

La distribución de las plagas dentro de los árboles y su movimiento a lo largo del día son factores a considerar para mejorar el muestreo de cada plaga y realizar las aplicaciones de insecticidas de forma más efectiva. Por ello, el **segundo objetivo** fue describir la **distribución espacial en el árbol a lo largo del día de las dos generaciones de ninfas de *P. kellyanus*** observadas durante el periodo en que el fruto es más susceptible. Al abordar este segundo objetivo también se observó que los frutos atacados por *P. kellyanus* tendían a caerse en mayor medida que los no atacados, por ello se decidió evaluar el **si el ataque de las ninfas de *P. kellyanus* tenía algún efecto en la caída prematura de frutos**.

A pesar de estar presente en varias zonas citrícolas, son escasos los estudios de eficiacia que comparan materias activas con diferente modo de acción y, además, evalúan sus efectos secundarios sobre la fauna auxiliar presente en los cítricos. Por todo ello, **el tercer objetivo** de esta tesis **fue determinar y comparar i) la eficacia de tres insecticidas con**

diferente modo de acción (clorpirifos, spinosad y spirotetramat) contra ninfas y adultos de *P. kellyanus* en campo; ii) su **persistencia** en caso de producirse un segundo ataque de *P. kellyanus* mientras el fruto es más susceptible y iii) su eficacia para disminuir el porcentaje de frutos dañados. Estos resultados permitirán recomendar el número de tratamientos necesarios cuando se empleen estos insecticidas. Finalmente, iv) se determinó los **efectos secundarios** de estos productos sobre fitoseidos en campo y sobre el coccinélido *Cryptolaemus montrouzieri* y el parasitoide *Aphytis melinus* en el laboratorio. Estos tres grupos de enemigos naturales son clave en el cultivo de los cítricos.

CAPÍTULO 3

Importance of the first and second attack of *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) nymphs: effect on fruit damage

3. Importance of the first and second attack of *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) nymphs: effect on fruit damage

Abstract

Pezothrips kellyanus (Bagnall) (Thysanoptera: Thripidae) has become a citrus pest in Spain. Nymphs of this thrips develop and feed on the surface of young fruits, generally under the calyces. This feeding habit produces rings of scar tissue around the apex as fruit matures. However, the number of attacks (thrip generations) are unknwon. In this study, we sampled two Navel orchards to determine the number of generations that attacked and caused damages. Moreover, we characterized the damages produced by both generations by measuring the diameter of the rings. The seasonal abundance of the nymphs on young fruits, as well, as the damages varied between orchards. Depending on the orchard, one or two generations (attacks) were observed on the young fruits. The second generation was detected between three and five weeks later than the first. Therefore, it is necessary sampling weekly from petal fall until 5 or 6 weeks later. Moreover, our data showed that when a second generation occurred the damages were more severe, suggesting that the second generation is more damaging. Finally, although there was a high variability in the diameter of the ring scar, this measure could be used to differentiate the damages produced by the first and second generation.

Keywords. Citrus, thrips, Kelly’s citrus thrips, seasonal abundance, damages

3.1 Introduction

During the last fifteen years, *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) has spread as a citrus pest in the Mediterranean basin and, nowadays, it is considered a pest also in Spain (Blank and Gill 1997, Webster et al. 2006, Conti et al. 2001, Varikou et al. 2002, Vassiliou 2007). In 2005, its presence was detected in some citrus orchards and, in 2008, damages produced by this thrips were recorded in some orchards from Valencia (Navarro et al. 2008a, Navarro et al. 2008b). Damage can be easily detected on the surface of mature fruits as rings of scarred tissue around the fruit apex. This damage does not affect the internal quality of fruit but it leads to economic losses due to reduced market value of the affected fruits (Varikou et al. 2002; Vassiliou 2010) (Figure 3.1).

Figure 3.1. Damages produced by nymphs of *P. kellyanus*.

Females of *P. kellyanus* lay eggs on citrus flowers and new fruitlets (Baker 2006). Then, nymphs of first and second stage are observed around the calix of these fruitlets, where they shelter and feed. Although first instar nymphs are transparent, the second instar nymphs are orange and can be easily identified. They can also be observed under the calyx and fruits in contact (Figure 3.2). Generally, it has been assumed that *P. kellyanus* nymphs cause damages from petal fall until five or six weeks later. However, any study has not clearly demonstrated it and, moreover, this period may vary among geographic regions (Vassiliou 2007; Navarro-Campos et al 2013).

Figure 3.2. *P. kellyanus* nymphs around the peduncle of young fruit.

In fact, the intensity and severity of the damages caused by *P. kellyanus* varies among years in Valencia. These differences are associated with temperature in winter and spring, as well as, the presence of other plants present in citrus orchards (Navarro-Campos et al. 2013). Moreover, some fruits present two ring scars suggesting that have been attacked at different moments during fruit growth. However, it is unknown whether these damages are produced by nymphs of the same generation. Finally, and more importantly, it is unknown whether one of these generation cause is more damaging. The economic threshold ranges between 5% and 10% of fruits attacked for Navel and Valencia citrus groups, respectively (Flint et al. 1991). A recent study stablishes this threshold at 12% of occupied fruits (Navarro et al. 2012a).

In this study, two Navel citrus orchards were sampled in two consecutive years to i) determine the seasonal abundance of *P. kellyanus* nymphs, those that cause damages, between petal fall and eight weeks later. Subsequently, in a citrus orchard where two generations of damaging nymphs were observed, we ii) determined and compared the damages produced by the first and second generation. Finally, we iii) determined whether damages caused by first and second generation can be distinguished by the diameter of the ring scar produced by *P. kellyanus*.

3.2 Material and Methods

3.2.1 Seasonal abundance of Pezothrips kellyanus nymphs and damages

3.2.1.1 Alzira orchard

This assay was conducted in a 16-years-old navel orange ‘Lane-late’ orchard [*Citrus sinensis* Blanco var. Navel Lane-Late grafted on Citrange ‘Carrizo’ (*Citrus sinensis* L. Osbeck × *Poncirus trifoliata* Blanco)] located near the town of Alzira (UTM: X = 725190.34; Y = 4339126.26) (Valencia, Spain) in 2009 and 2010. The orchard had 1.4 ha and the planting pattern was 6 x 5 m. It was drip irrigated and the naturally-occurring cover crop was mowed annually at the beginning of spring.

At petal fall, the population density of nymphs was over the economic threshold both years. 10 trees were sampled and selected according to their similar infestation level by *P. kellyanus* nymphs (15-25% occupied fruits) to evaluate the seasonal abundance of *P. kellyanus* nymphs on fruits. 32 fruits per tree (eight per orientation) were sampled the day that the economic threshold (5% of fruits occupied by nymphs of *P. kellyanus*) was exceeded, two days later and then weekly until the end of the study. We determined the presence of *P. kellyanus* nymphs on each fruit. To determine the percentage of damaged fruit at harvest, the same number of fruits in the same trees were sampled and classified. Sampled fruits were classified as fruit without damage, fruit with severe damage (one or two scarring around the calyx) (Figure 3.3) and fruit with slight damage (scarring around the calyx not complete) (Figure 3.4).

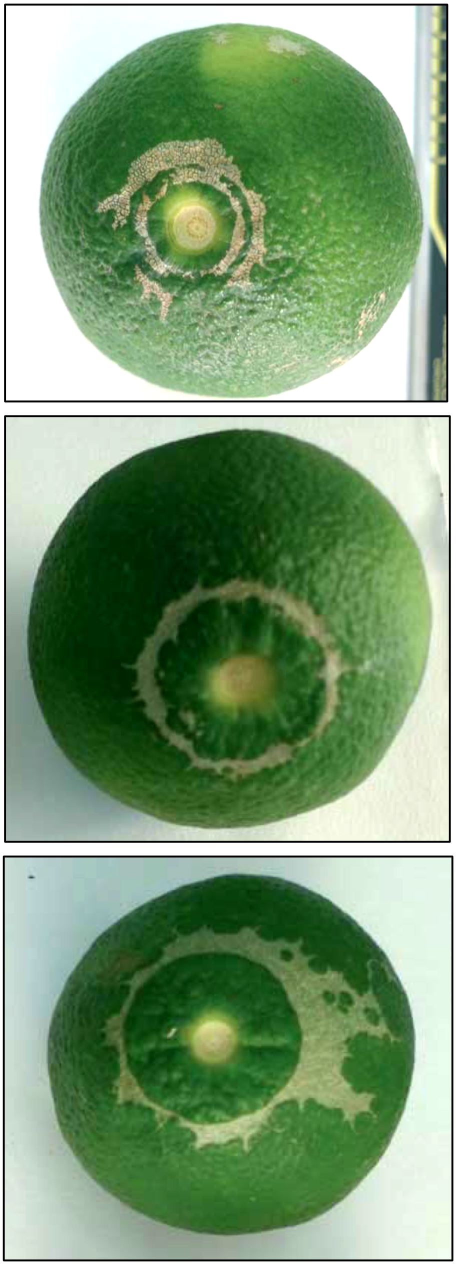


Figure 3.3. Severe damage observed on fruits.

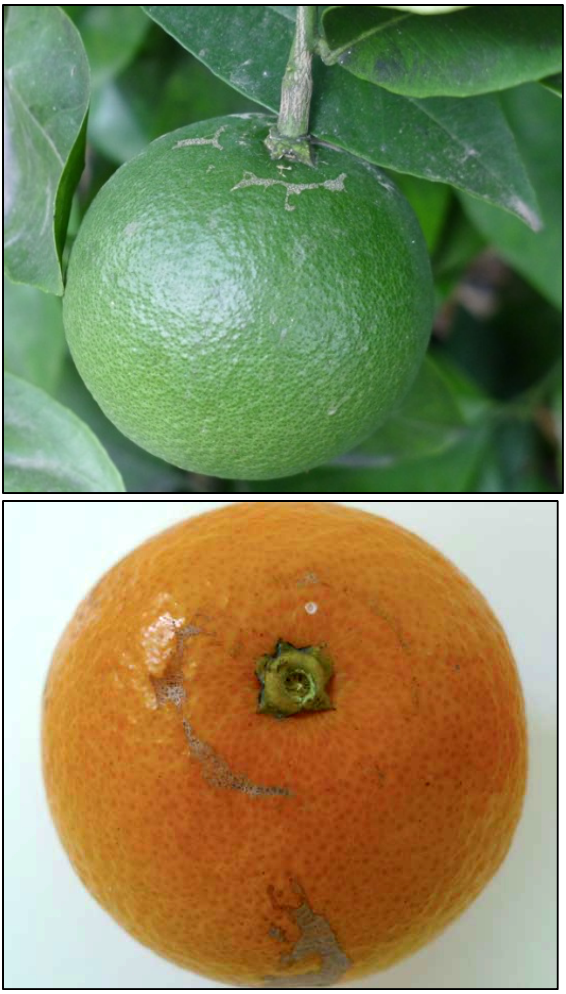


Figure 3. 4. Slight damage observed on fruits.

3.2.1.2 Tabernas de la Valldigna orchard .

This assay was conducted in a 10-years-old navel orange 'Lane-late' orchard [*Citrus sinensis* Blanco var. Navel Lane-Late grafted on Cleopatra mandarin (*Citrus reshi* Hort. ex. Tan.)] located near the town of Tavernes de la Valldigna (hereinafter Tavernes) (UTM: X = 739997.68; Y = 4328225.46) (Valencia, Spain) in 2010 and 2011. The orchard had 3.5 ha and the planting pattern was 6 x 4 m. It was drip irrigated and the naturally occurring cover crop was mowed annually at the beginning of spring. The population density of nymphs exceeded the economic threshold (5%) some weeks after petal fall both years (on May 31 in 2010 and May 16 in 2011). Then, 40 trees were randomly selected and sampled in 2010 and 10 trees in 2011. The percentage of occupied fruits and damage fruits was determined as above sampling 32 fruits (eight per orientation) per tree.

3.2.2 Influence of the first and second generation of *Pezothrips kellyanus* nymphs on damages

To determine the influence of the first and second generation of *P. kellyanus* nymphs on the damages produced, we sampled the orchard of Tavernes de la Valldigna in 2011. There, 217 fruits were marked and daily sampled during the first and second generation of *P. kellyanus* nymphs and the presence of *P. kellyanus* nymphs was determined. Fruits were marked with a transparent plastic ring 1.5 cm in diameter. In total, 76 and 141 fruits were sampled in the first and second generation (no signs of damage after the first generation). In November, the same fruits were classified as fruits without damage, with severe damage (one or two circular rings) and light damage (not complete ring).

3.2.3 Diameter of the ring scar caused by *Pezothrips kellyanus*

The diameter of ring scar and the diameter of the fruit were measured in Tavernes de la Valldigna in 2010 in 40 trees to correlate both measures. A total of 20 damaged fruits per tree were sampled with a caliper. The following year, we used the fruits marked in the above secction (3.2.2) to determine whether the diameter of the ring scar was different for the first and second generation.

3.2.4 Data analysis

Normality and homogeneity of variance were tested using Kolmogorov-Smirnov and Cochran's tests respectively. Data were transformed (angular transformation for percentage data) if needed. Influence of first and second generation of *Pezothrips kellyanus* nymphs on damages and diameter produced by *P. kellyanus* were analyzed by one-way ANOVA followed by Turkey hoc tests for multiple comparisons.

3.3 Results and Discussion

3.3.1 Seasonal abundance of *Pezothrips kellyanus* nymphs

The percentage of occupied fruits by *P. kellyanus* nymphs was higher than 18% after petal fall both years in the orchard from Alzira (Figure 3.5). One week later, the percentage decreased below 5% both years and it did not exceed this level during the rest of the sampling period. Therefore, there was only one attack of *P. kellyanus* nymphs in this orchard. The damage observed at harvest was similar both years (*P* =

0,81; F $_{1, 34}$= 0,057) and there were not significant differences between the percentage of fruits slightly and severely damaged (Table 3.1).

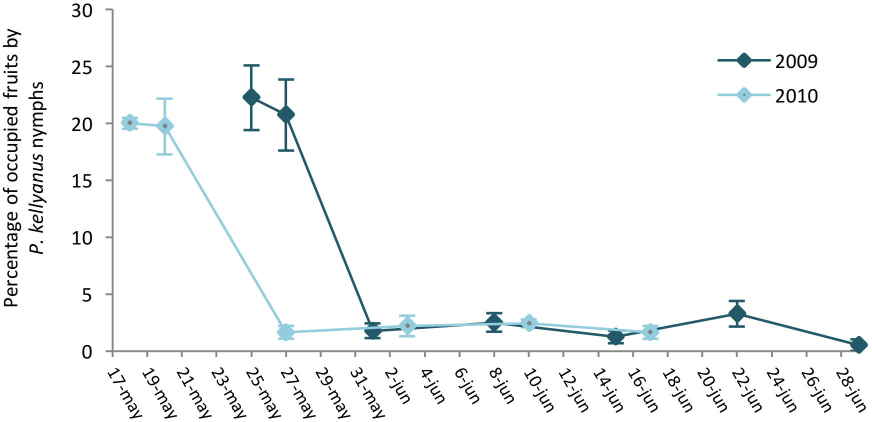


Figure 3.5. Seasonal abundance of *Pezothrips kellyanus* nymphs on young fruits in a citrus (Navel) orchard from Alzira in 2009 and 2010. Sampling started after petal fall (90% of petals).

Table 3.1. Percentage of fruits with slight and severe damages produced by *P. kellyanus* nymphs in two navel orchards located in Forn de Carrascosa and Tavernes de la Valldigna.

Orchard	Year	Slight damages	Severe damages	Statistics
Alzira	2009	25.94 ± 1.41	21.56 ± 3.02	F $_{1, 14}$ = 1.719; *P* = 0.211
	2010	24.00 ± 1.52	20.42 ± 2.56	F $_{1, 19}$ = 1.448; *P* = 0.245
Tavernes de la Valldigna	2010	26.88 ± 1.36 b	39.55 ± 2.58 a	F $_{1, 78}$ = 18.93; $P \leq 0.0001$
	2011	15.94 ± 1.71 b	30.94 ± 2.57 a	F $_{1, 78}$ = 23.62; *P* = 0.0001

Different letters show differences among kind of damage produced ($P < 0.05$).

The percentage of occupied fruits by *P. kellyanus* nymphs after petal fall did not exceed 5% any year in the orchard from Tavernes. Several weeks after, this threshold was exceeded and we, therefore, started to sample (Figure 3.6). In 2010, this percentage of occupancy (~ 15%) was higher than in 2011 (~ 6%). This percentage decreased below 5% one week later and again increase and exceed 5% three weeks after the first maximum, suggesting that a second generation of nymphs attacked the fruit. In this orchard, damages observed at harvest were higher in 2010 than in 2011 (P = 0.0053; $F_{1,\ 98}$ = 8.14) and , moreover, there were significant differences between the percentage of fruits slightly and severely damaged (Table 3.1).

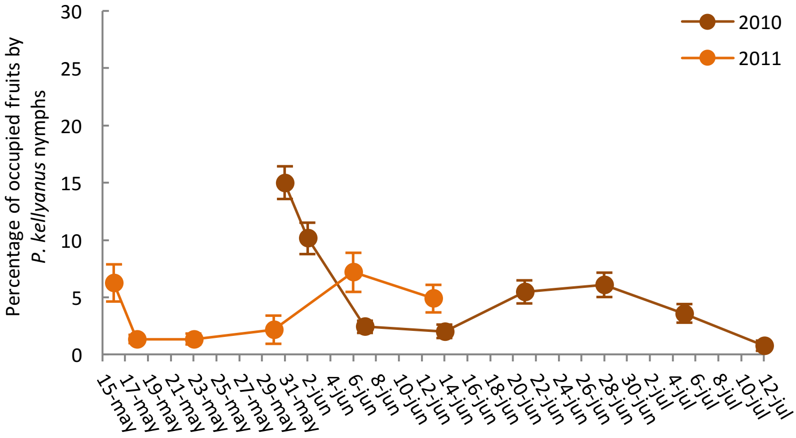


Figure 3.6. Seasonal abundance of *Pezothrips kellyanus* nymphs on young fruits in a citrus (Navel) orchard from Tavernes in 2010 and 2011. Sampling started after petal fall (90% of petals).

These results, based on sampling *P. kellyanus* nymphs on fruit, indicated that one or two generations of nymphs can attack the fruit after petal fall depending on the orchard but not on the year. In Alzira, only one generation attacked the fruits after petal fall. The "5% threshold" was exceeded during one week, remaining low the rest of the sampling period. In Tavernes, however, the percentage of occupied fruits after petal fall was lower than in Alzira orchard but a second attacked was detected three weeks after the first attack. This variability between orchards underlines the importance of sampling weekly from petal fall until 5 or 6 weeks later.

3.3.2 Influence of the first and second generation of *Pezothrips kellyanus* nymphs on damages

Fruit occupied only by the first generation showed mostly slight damage (~ 64%), whereas fruit occupied by the second generation had a similar percentage of slight (~ 36) and severe damage (~ 33%) (Table 3.2). Fruit occupied by both generations showed 100% of severe damage. Finally, fruits not occupied by *P. kellyanus* nymphs during our observations showed a 31% of slight damage.

Table 3.2. Influence of the first and second generation of *Pezothrips kellyanus* nymphs on the percentage of fruit damaged in a citrus orchard (Navel) from Tavernes de la Valldigna in 2011.

Fruit occupied by nymphs	Undamaged fruit (%)	Fruit with slight damage (%)	Fruit with severe damage (%)	Statistics
1st Generation	36.4 ± 15.2 bB	63.6 ± 15.2 a A	0.0 ± 0.0 cC	$F_{2, 10} = 6.61$; $P = 0.0042$
2nd Generation	30.3 ± 5.7 aB	36.4 ± 6.0aB	33.3 ± 5.8 aB	$F_{2, 65} = 0.27$; $P = 0.7642$
1st and 2nd Generation	0.0 ± 0.0 bC	0.0 ± 0.0 bC	100.0 ± 0.0 aA	
Not occupied	65.5 ± 9.0 aA	31.0 ± 8.7 bB	3.4 ± 3.4 cC	$F_{2, 28} = 17.17$; $P \leq 0.0001$
Statistics	$F_{3, 108} = 4.56$; $P = 0.048$	$F_{3, 108} = 1.89$; $P = 0.1361$	$F_{3, 108} = 9.33$; $P \leq 0.0001$	

Different lower case letters show differences among the intensity of the damage produced for each generation ($P < 0.05$).

Different capital letters show differences among fruits occupied by different *P. kellyanus* generations for each type of damage ($P < 0.05$).

Our data suggests that the second generation of *P. kellyanus* is more damaging than the first. First of all, in Alzira with only one generation, the percentage of damaged fruit was 50% with a maximum of fruit ocupied with nymphs of ~20%. In Tavernes, where two generations were observed, the percentage of damaged fruit reached 70% in 2010 even though the percentage of occupied fruit was much lower than in Alzira. In our second assay, where we depected the intesity of the damages, we observed that fruits occupied by first generation have mainly slight damage, whereas those occupied by the second generation have a similar percentage of slight and severe damages. These results reinforce that the second generation of *P. kellyanus* nymphs, although less intense, is more harmful than the first generation.

3.3.3 Diameter of the ring scar caused by *Pezothrips kellyanus*

The diameter of the ring scar produced by *P. kellyanus* nymphs was not correlated with the diameter of the fruit ($F_{1,\ 798}$ = 0.010, P = 0.98). Ring scars ranged from a minimum of 7 mm to a maximum of 34 mm, being the mean 16.09 ± 0.16 mm (Figure 3.7).

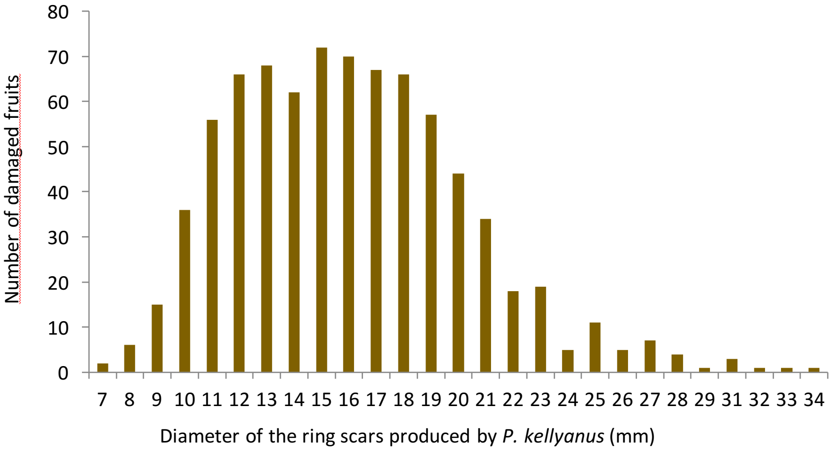


Figure 3.7. Diameter of the ring scar produced by *Pezothrips kellyanus* nymphs in a citrus orchard (Navel) from Tavernes de la Valldigna in 2010.

When we compared the diameter of the ring scar produced by the first and second generation, the former (18.09 ± 0.72 mm) produced a significantly larger ring scar than the latter (13.58 ± 0.43 mm) ($F_{1,\ 76}$ = 32.96, P <0.0001) (Figure 3.8).

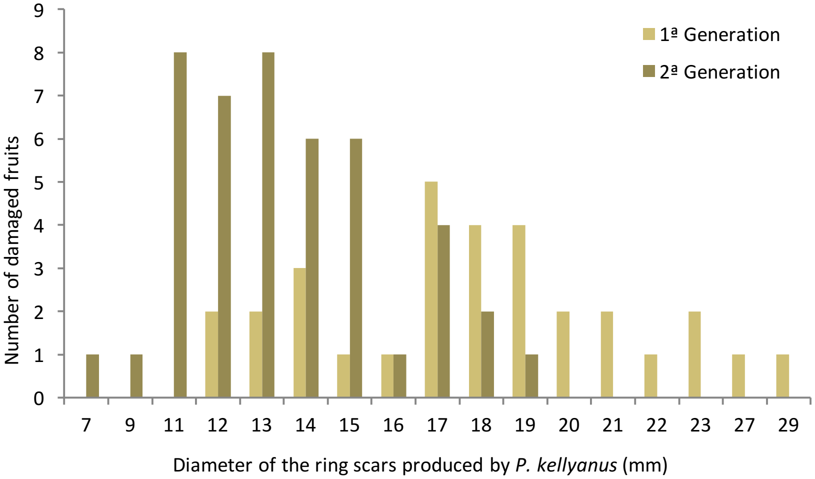


Figure 3.8. Diameter of the ring scar produced by the first and second generation of *Pezothips kellyanus* in a citrus orchard (Navel) from Tavernes de la Valldigna in 2011.

During the sampling of the first year, we observed a great variability in the size of the ring scars (7-34 mm). We hypothesized that it was due to the size of the fruit but we could not find any relationship. The following year, we could demonstrated that this variability can be partially explained by the attack of different generations of *P. kellyanus*. The diameter of the rings caused by the first generation was about 5 mm higher than those of the second. These data suggest that the diameter of damage could be used to determine the virulence of the first and second generation of *P. kellyanus* in future studies.

In conclusion, our study shows that the number of generations of *P. kellyanus* that can attack and damage fruit varies mainly among orchards and between one and two generations can occur. Therefore, it is necessary sampling weekly from petal fall until six weeks later. In addition, our data suggest that when fruits are attacked by a second generation, this is more damaging than the first one.

Acknowledgments

We are grateful to Bautista Domènech and Bernardo Villalba for allowing us to sample their orchards. We also thank P. Bru, H. Montón, C. Monzó, B. Sabater, E. Llácer, Khaled Abbas, F. Gómez-Marco (IVIA) for their technical assistance. This research was partly funded by the Spanish Ministry of Science and Innovation (project: AGL2011-30538-C03-02) and by the Conselleria d'Agricultura, Pesca i Alimentació from Generalitat Valenciana.

CAPÍTULO 4

Within-tree and temporal distribution of *Pezothrips kellyanus* nymphs in citrus canopies and their influence on premature fruit abscission

Within-tree and temporal distribution of *Pezothrips kellyanus* nymphs in citrus canopies and their influence on premature fruit abscission

Planes L, Catalán J, Urbaneja A and Tena A. 2014. Within-tree and temporal distribution of *Pezothrips kellyanus* nymphs in citrus canopies and their influence on premature fruit abscission. Environmental Entomology, 43 (3) : 689-695.

Abstract

Pezothrips kellyanus (Bagnall) (Thysanoptera: Thripidae) has recently become a pest of citrus whose nymphs feed on the surface of young fruitlets. This feeding habit causes patches or rings of tissue scar around the apex as fruit mature. Currently, little is known about the distribution of *P. kellyanus* nymphs. Further knowledge would allow the development of an appropriate sampling protocol and targeted pesticide application. In our first experiment, the abundance of first- and second-generation *P. kellyanus* nymphs was surveyed in a citrus orchard at different times of day to characterize their spatial and temporal distributions. The distribution of damaged fruit was also measured at harvest. Our results showed that P. kellyanus nymphs tended to be present in the upper half of the canopy and mainly damaged the fruit located in this area of the canopy. However, *P. kellyanus* nymphs were uniformly distributed among the four cardinal directions of the canopy and throughout the day. Consequently, cardinal direction and time of the day seem to be less important when developing a sampling plan or in improving targeting or timing of insecticidal spray applications. In our second experiment, we tracked the presence of P. kellyanus nymphs in labeled fruit daily. These data were used to determine how many days the nymphs occupied a fruit and to relate occupancy and premature fruit abscission. The nymphs of P. kellyanus remained on the same fruit for only one day. The rate of fruit abscission in June was significantly higher in fruit occupied by first-generation *P. kellyanus* nymphs than in non-occupied fruit.

Keywords: citrus, IPM, thrips, Kelly’s citrus thrips, fruit abscission.

4.1 Introduction

Pezothrips kellyanus (Bagnall) (Thysanoptera: Thripidae) has recently become a pest of citrus in New Zealand, southern Australia and the Mediterranean basin (Stevens et al. 1998, Varikou 2002, Baker 2004, Vassiliou 2007, Navarro et al. 2008a). Females lay eggs in citrus flowers, mainly on petals, and fruit (Vassiliou 2007). Nymphs develop on fruit, and they are commonly observed feeding on the surface of young fruitlets under the calyces (Teulon and Penman 1991). This feeding habit causes patches or rings of scar tissue around the apex as fruit mature (Jamieson and Stevens 2006, Webster et al. 2006). Although this thrips species does not affect the internal quality of the fruit, such damage leads to economic losses due to the reduced market value of the affected fruit (Varikou 2002, Vassiliou 2010). This damage is severe on lemons and grapefruit, particularly on navel varieties that retain their sepals, under which thrips nymphs shelter and feed (Marullo 1998, Mound and Jackman 1998, Baker et al. 2011).

In the Mediterranean basin, *P. kellyanus* have up to six generations per year (Vassiliou 2007). In citrus orchards, *P. kellyanus* adults peak during the flowering period in spring (Vassiliou 2007, 2010). Nymphs feed on new fruitlets and damage them from petal fall (anthesis) until five to six weeks later. Generally, one or two generations of nymphs attack fruit during this period (unpublished data). Navarro-Campos et al. (2012a) demonstrated that fruit damage is strongly correlated with the percentage of fruit occupied by immature *P. kellyanus*, which also has also been recorded in other citrus thrips such as *Scirtothrips citri* (Moulton) (Thysanoptera: Thripidae) (Tanigoshi and Moffitt 1984, Schweizer and Morse 1997). Based on the percentage of fruit occupied by *P. kellyanus* nymphs, Navarro-Campos et al. (2012a) calculated the economic injury level and the environmental economic injury level (using the organophosphate insecticide chlorpyrifos) as 7 and 12%, respectively. Therefore, insecticide treatments would be necessary if more than 12% of fruit are infested by immature thrips. This sampling method and similar thresholds have been used to sample other thrips species in citrus. For example, levels of 5-10% for navel and 20% for Valencia fruit have been proposed as thresholds to apply management practices for *S. citri* in California (IPM for citrus 1991).

A step towards an efficient and practical sampling method is to determine an appropriate sample unit. Knowledge of factors such as the within-tree distribution and diurnal distribution of *P. kellyanus* is important not only to estimate accurate thrips populations but also for targeting pesticide applications if chemical control is attempted. This might be especially important for *P. kellyanus* because the few available references show that adults tend to aggregate for mating (Mound and Jackman 1998) and concentrate in the outer northern and eastern sides of citrus canopies in Cyprus (Vassiliou 2010). However, the distribution of nymphs, which causes damages, within the tree and throughout the day is unknown. This might be important because nymphs move from fruit to fruit within the plant to obtain new food or to shelter from natural enemies and adverse climatic conditions (mainly high sunlight and low relative humidity) (Kirk 1985a). These movements may cause significant differences in the density of *P. kellyanus* among canopy sites within only a few hours (Kirk 1985b).

In addition to damage to the fruit, *P. kellyanus* might also cause fruit abscission in June. Citrus trees have a self-regulatory mechanism whereby they shed part of their fruit load during the period of cell division, i.e., between anthesis and June (Gómez-Cadenas et al. 2000, Agustí 2000). This ensures that more fruit than can be supported under prevailing environmental conditions are not retained by the tree (Bangerth 2000). Given that *P. kellyanus* nymphs feed on fruit during this period, their feeding habits might cause fruit abscission. However, to our knowledge, this hypothesis has never been tested for *P. kellyanus* or other citrus thrips species.

The purpose of this study is twofold: i) to describe the spatial and diurnal distribution of first- and second-generation *P. kellyanus* nymphs and the spatial distribution of the damage; ii) to determine the number of days that a fruit is occupied by the nymphs of *P. kellyanus* and to relate the residency time of *P. kellyanus* nymphs on fruit with fruit abscission rates in June. The first purpose aids in designing better sampling protocols and targeting pesticide applications and the second purpose will provide additional information about the economic importance of *P. kellyanus* as citrus pest.

4.2 Materials and Methods

4.2.1 Citrus orchard

All assays were conducted in a ten-year-old navel orange 'Lane-late' orchard [*Citrus reticulate* Blanco var. Navel Lane-Late grafted on Cleopatra mandarin *(Citrus reshi* Hort. ex. Tan.)] in Tavernes de la Valldigna (UTM: X = 739997.68; Y = 4328225.46) (Valencia, Spain). This orchard was 3.5 ha, and the planting pattern was 6 x 4 m. It was drip irrigated, and the cover crop was mowed annually in spring. Assays were carried out in a plot of 1 ha with eight rows of trees. Trees in the two rows bordering the plot on all sides were excluded from the experiment to eliminate edge effects. Trees were not sprayed with insecticides during the two years that the assay was conducted.

4.2.4 Within-tree Distribution

To determine the within-tree distribution of *P. kellyanus* nymphs, the percentage of fruit occupied by *P. kellyanus* nymphs (either on the fruit or under the calix) was measured weekly from petal fall until the end of the second generation in 2010 and 2011 following the guidelines of IPM for citrus in Valencia Region (Urbaneja et al. 2015). One day after the percentage of occupied fruit exceeded the economic injury level (5%) (Urbaneja et al. 2015) (May 31th and June 23rd in 2010; May 16th and June 6th in 2011), 40 and 25 trees of the plot were randomly selected and labeled in 2010 and 2011, respectively. We observed eight fruits from each direction (N, S, E and W) of the tree canopy, four above and four below a height of 1.5 m (32 fruits per tree) to determine the spatial distribution of *P. kellyanus* nymphs within the tree. Sampling occurred between 8:00 and 10:00.

The trees were sampled again on September 23rd in 2010 and September 8th in 2011 to determine the spatial distribution of damaged fruit within the tree. Following the same sampling procedure, 32 fruits from each labeled tree were observed. A fruit was recorded as damaged when it had a complete ring scar (Navarro-Campos et al. 2012a, 2013).

4.2.3 Diurnal distribution

The diurnal distribution of *P. kellyanus* nymphs was determined during the first generation of nymphs that attacked the fruit in 2010 and during the first and second generation in 2011. Both years, we observed eight fruits from each direction (N, S, E and W) of the tree canopy (four above and four below 1.5 meters) from each tree (32 fruits per tree) three times per day (08-10:00, 13-15:00, 19 -21:00). In 2010, we sampled fruit one day after the percentage of occupied fruit exceeded the economic injury level (5%) (May 31th), the same ten trees were sampled. In 2011, we sampled the percentage of occupied fruit three times per day (08-10:00, 13-15:00, 19-21:00) during four consecutive days of each generation. One, two, three and four days after the percentage of fruit occupied exceeded the economic injury level (5%) (May 16th and June 6th), the same 25 trees were sampled. We observed eight fruits from each direction (N, S, E and W) of the tree canopy (four above and four below 1.5 meters) from each tree (32 fruits per tree).

4.2.4 Fruit occupancy time

To determine the number of days that a fruit was occupied by nymphs of *P. kellyanus* (those that are used to determine the environmental economic injury level), a total of 217 occupied fruits were observed daily until the nymphs left the fruit. Fruits were labeled using a transparent plastic ring (1.5 cm diameter). Seventy-six fruits were labeled on May 16th (first generation), and 141 fruits were labeled on June 6th (second generation). A maximum of 18 and a minimum of four fruits were sampled per tree with a mean of 9.04 ± 0.86 fruits per tree. Observations were made between 10:00 and 12:00.

4.2.5 Influence of *Pezothrips kellyanus* on premature fruit abscission

To determine the influence of *P. kellyanus* on premature fruit abscission, 188 fruits were tracked daily for one week during the first generation of nymphs in 2011. We recorded the presence of *P. kellyanus* nymphs. Fruits were labeled on the first day (May 16th, 2011) using a transparent plastic ring (1.5 cm diameter). A maximum of 27 and a minimum of three fruits were sampled per tree. Observations were made between 10:00 and 12:00.

Fruit were sampled on June 6th (the beginning of the second generation of nymphs) to count the number of fruit that had dropped and to test whether the attack of the first generation of *P. kellyanus* nymphs had affected fruit abscission in June.

4.2.6 Meteorological data

Air temperature, relative humidity and solar radiation data were obtained from a meteorological station situated ~0.5 km away from the orchard where the study was carried out (IVIA 2012).

4.2.7 Data analysis

Statistical comparisons of within-tree distribution and diurnal distribution were performed using ANOVA, and the means were compared using the Tukey test ($P < 0.05$). Percentage data were subjected to an angular transformation to approximate a normal distribution before analysis. The Pearson's chi-square analysis was used to determine whether the attack of the first generation of *P. kellyanus* nymphs affected fruit abscission in June.

4.3 Results

4.3.1 Within-tree distribution

The first generation of *P. kellyanus* nymphs in 2010 was not uniformly distributed within the tree. Significant differences were found between the upper and lower halves of the canopy ($F = 5.73$; df = 1, 312; $P = 0.02$) and among the four cardinal directions ($F = 7.23$; df = 3, 312; $P < 0.0001$), the interaction between these factors was not significant ($F = 1.22$; df = 3, 312; $P = 0.3$) (Table 4.1). During the second generation, the percentage of fruit occupied by nymphs was also significantly higher in the upper than in the lower half ($F = 16.31$; df = 1, 312; $P < 0.0001$), but there were not significant differences among directions ($F = 0.53$; df = 3, 312; $P = 0.66$) and the interaction between these factors was not significant ($F = 0.63$; df = 3, 312; $P = 0.59$). Similarly, the percentage of damaged fruit was significantly higher in the upper half of the canopy ($F = 51.83$; df = 1, 312; $P < 0.0001$), whereas there were no significant differences among directions ($F = 0.73$; df = 3, 312; $P = 0.54$) and the interaction between these factors was not significant ($F = 0.89$; df = 3, 312; $P = 0.45$).

The first- and second-generation *P. kellyanus* nymphs in 2011 were uniformly distributed within the tree, there were no significant differences between the upper and lower parts of the canopy (first-generation: $F = 0.87$; df = 1, 192; $P = 0.35$; second generation: $F = 0.38$; df = 1, 192; $P = 0.54$) or among cardinal directions (first-generation: $F = 0.94$; df = 3, 192; $P = 0.42$; second generation: $F = 1.11$; df = 3, 192; $P = 0.35$) and the interaction between these factors was not significant (first-generation: $F = 0.38$; df = 3, 192; $P = 0.76$;

second generation: $F = 0.73$; df = 3, 192; $P = 0.54$) (Table 1). However, the percentage of damaged fruit was significantly higher in the upper half of the canopy ($F = 6.49$; df = 1, 192; $P < 0.0001$). There were no significant differences among directions ($F = 2.91$; df = 3, 192; $P = 0.62$) and the interaction between these factors was not significant ($F = 0.45$; df = 3, 192; $P = 0.72$).

Table 4. 1. Mean percentage of occupied and damaged fruit by *Pezothrips kellyanus* nymphs in a navel orange orchard in 2010 (number of sampled trees; n = 40) and 2011 (n = 25).

º	Height	Direction	Occupied fruit		Damaged fruit
			1st generation	2nd generation	
2010	Upper	N	21.9 ± 4.2	14.1 ± 4.0	51.9 ± 4.9
		S	23.8 ± 4.5	7.7 ± 3.4	53.2 ± 6.0
		E	20.0 ± 3.8	6.3 ± 3.7	49.4 ± 5.6
		W	6.3 ± 2.0	10.0 ± 3.2	60.6 ± 5.3
		Average	18.0 ± 3.9	9.5 ± 3.6	53.8 ± 5.4
	Lower	N	20.6 ± 4.3	1.3 ± 1.3	29.5 ± 5.3
		S	13.1 ± 3.3	0.0 ± 0.0	23.1 ± 4.9
		E	8.8 ± 2.5	3.8 ± 2.1	24.4 ± 4.1
		W	5.6 ± 2.3	0.0 ± 0.0	25.6 ± 5.2
		Average	12.0 ± 3.3	1.2 ± 1.2	25.6 ± 4.8
Average			15.0 ± 3.6	5.4 ± 2.8	39.7 ± 5.6
2011	Upper	N	9.0 ± 4.8	1.5 ± 1.1	35.4 ± 6.6
		S	7.0 ± 3.1	2.0 ± 1.6	31.0 ± 5.6
		E	14.0 ± 3.6	7.5 ± 3.1	33.0 ± 5.1
		W	8.0 ± 3.5	4.5 ± 1.6	25.0± 4.8
		Average	9.5 ± 3.8	3.8 ± 2.0	31.1 ± 5.5
	Lower	N	5.0 ± 2.5	0.5 ± 0.5	8.3± 2.5
		S	9.0 ± 2.5	0.5 ± 0.5	7.5 ± 3.4
		E	11.0 ± 3.8	2.5 ± 1.3	10.0 ± 3.2
		W	5.0 ± 2.5	1.0 ± 1.0	8.3 ± 2.8
		Average	7.5 ± 3.1	1.1 ± 0.9	8.4 ± 3.0
Average			8.5 ± 3.4	2.5 ± 1.6	19.9 ± 5.0

4.3.2 Diurnal distribution

Although temperature, light intensity and relative humidity differed among the time of the day (Table 4.2), the percentage of fruit occupied by *P. kellyanus* nymphs did not differ significantly throughout the day in both years and generations (Table 3 and Table 4).

Table 4. 2. Mean temperature, relative humidity and solar radiation at different times of day (7 - 9:00, 13 - 15:00 and 19 - 21:00) during the first generation of *Pezothrips kellyanus* nymphs in 2010 and during the first and second generations in 2011.

Meteorological factors	**2010**			**2011**					
	1st generation			**1st generation**			**2nd generation**		
	8:00	14:00	20:00	8:00	14:00	20:00	8:00	14:00	20:00
Temperature (ºC)	29.3	31.5	26.0	17.6	17.6	15.6	19.2	21.2	18.2
Relative humidity (%)	38.5	36.9	53.7	48.2	54.3	63.5	74.0	64.6	77.4
Solar radiation (w/m^2)	596.8	830.6	2.8	362.5	253.1	0.8	370.5	569.0	0.2

During the first generation of 2010, there was a significant interaction between the time of the day and cardinal direction (Table 4). This interaction was due to the increase of the percentage of occupied fruits in the North side of the canopy at 20:00 (Table 3). None of the interactions between the time of the day and other factors was significant in either generation in 2011.

Table 4. 3. Percentage of fruit occupied by *Pezothrips kellyanus* nymphs at different times of day (8:00, 14:00 and 20:00) during the first generation in 2010 (number of sampled trees; n = 10) and during the first and second generations in 2011 (n = 25).

Year	**Height**	**Direction**	**1st generation**			**2nd generation**		
			8:00	**14:00**	**20:00**	**8:00**	**14:00**	**20:00**
2010	Upper	N	17.5 ± 5.3	12.5 ± 4.2	32.5 ± 10.6			
		S	20.0 ± 9.7	32.5 ± 9.9	17.5 ± 6.5			
		E	20.0 ± 8.2	15.0 ± 6.7	15.0 ± 6.7			
		W	5.0 ± 3.3	10.0 ± 5.5	2.5 ± 2.5			
	Lower	N	20.0 ± 8.2	7.5 ± 3.8	27.5 ± 10.8			
		S	10.0 ± 5.5	17.5 ± 7.5	15.0 ± 8.5			
		E	17.5 ± 7.5	2.5 ± 2.5	5.0 ± 3.3			
		O	10.0 ± 5.5	0 ± 0	5.0 ± 5.0			
	Average		15.0 ± 2.4	12.2 ± 2.2	15.0 ± 2.7			
2011	Upper	N	2.5 ± 1.3	2.5 ± 1.8	3.3 ± 1.8	4.2 ± 2.6	2.5 ± 1.8	1.2 ± 1.2
		S	1.7 ± 1.1	1.7 ± 1.1	1.7 ± 1.1	2.5 ± 1.8	0.0 ± 0.0	1.2 ± 1.2
		E	2.5 ± 1.3	4.2 ± 2.2	3.3 ± 2.2	10.0 ± 3.7	3.3 ± 1.8	7.5 ± 3.3
		W	0.8 ± 0.8	0.0 ± 0.0	0.8 ± 0.8	3.3 ± 2.5	2.5 ± 1.8	7.5 ± 3.3
	Lower	N	0.0 ± .0.0	0.8 ± 0.8	0.8 ± 0.8	0.0 ± 0.0	1.7 ± 1.1	0.0 ± 0.0
		S	0.8 ± 0.8	0.8 ± 0.8	0.8 ± 0.8	1.7 ± 1.1	1.7 ± 1.7	2.5 ± 1.7
		E	0.8 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 1.1	1.7 ± 1.7	6.2 ± 3.4
		O	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 1.7	0.8 ± 0.8	2.5 ± 2.5
	Average		1.15 ± 0.32	1.25 ± 0.42	1.35 ± 0.43	3.12 ± 0.78	1.77 ± 0.51	3.59 ± 0.87

Table 4. 4. Analysis of variance of the diurnal distribution of *Pezothrips kellyanus* nymphs.

Source	df	2010		2011			
		1st generation		1st generation		2nd generation	
		F	*P*	*F*	*P*	*F*	*P*
Time	2	0.56	0.57	0.08	0.93	1.78	0.17
Height	1	3.46	0.06	14.36	0.0002	5.79	0.016
Direction	3	5.58	0.001	2.46	0.06	4.01	0.008
Time* Height	2	0.86	0.42	0.07	0.93	1.28	0.28
Time*Direction	6	2.49	0.02	0.14	0.99	0.96	0.45
Height *Direction	3	0.49	0.69	1.76	0.15	1.35	0.26
Time* Height *Direction	6	0.28	0.28	0.26	0.95	0.59	0.74

4.3.3 Fruit occupation time

The mean time that fruit were occupied by *P. kellyanus* nymphs after their detection was 1.37 ± 0.048 (n = 217) days, and 72.8% of fruit were occupied only one day after the detection of nymphs (Figure 4.1). The maximum time that a fruit was occupied was five days.

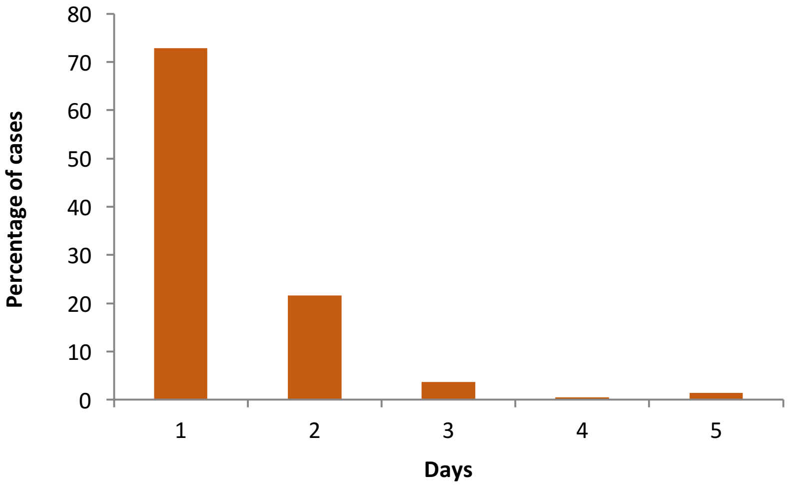


Figure 4. 1. Number of days that *Pezothrips kellyanus* nymphs occupied a fruit after their detection in a navel orange orchard.

4.3.4 Influence of *Pezothrips kellyanus* on fruit abscission

In June, the probability of premature fruit abscission was significantly higher in fruit occupied by first-generation *P. kellyanus* nymphs (48 out of 70; 68.6 %) than in non-occupied fruit (40 out of 118; 33.9 %) (χ^2=21.21; $P < 0.001$).

4.4 Discussion

Our data showed that *P. kellyanus* nymphs tended to occur in the upper half of the canopy and to damage the fruit located in this area of the canopy. Sixty-eight percent and 79.4% of the damaged fruit was located in the upper half in 2010 and 2011, respectively. Information regarding the distribution of *P. kellyanus* in citrus canopies, which is needed to improve management, is scarce because it is a relatively new international citrus pest. In general, immature thrips tend to develop in the upper half of the plants. For example, immature *Scirtothrips dorsalis*

Hood (Thysanoptera: Thripidae) on pepper and *Frankliniella tritici* (Fitch) (Thysanoptera: Thripidae) on cotton are more abundant in the upper half of their host plant (Seal et al. 2006, Osekre et al. 2009). Pankeaw et al. (2011) studied the distribution of damaging thrips in the mangosteen canopy based on visual assessment of scarring on the fruit surfaces. They also concluded that immature thrips are more abundant in the upper halves of the canopy than in the lower halves. It has been hypothesized that the greater occupancy of flowers and fruit in the upper half of plants is because the movement of adult thrips within a field occurs just above the top of the crop (Gillespie and Vernon 1990). As adults land, they tend to lay their eggs on the upper half of plants. This hypothesis is based on the higher number of adults collected in sticky traps located just above the crop canopy (Brødsgaard 1989, Gillespie and Vernon 1990). Conversely, the number of immature tomato thrips (*Frankliniella occidentalis* (Pergande), *F. fusca* (Hinds) and *F. tritici*) was greater on flowers in the lower half rather than in the upper half of tomato plants (Salguero-Navas et al. 1991). Authors suggested that it could be due to poorer coverage of insecticides in the lower half of the plants.

Contrary to expectations, the damage produced by *P. kellyanus* nymphs was equally distributed among the four directions of the canopy, and we could not detect any clear pattern of distribution for the nymphs. Although, to our knowledge, the distribution of nymphs and damage has not been studied before, we expected that nymphs, and consequently the damage, would be located mostly in the eastern and northern sides of the canopy for two reasons. First, the flight activity of thrips is strongly influenced by wind direction and the prevailing wind is from the east in spring and summer in eastern Spain (IVIA 2012). Second, thrips shelter from direct sunlight (Lewis 1997), which comes mainly from the south at this latitude. In Cyprus, *P. kellyanus* adults follow this pattern (Vassiliou 2008, 2010). However, the distribution of immatures and damage was not measured in these studies.

The percentage of fruit occupied by *P. kellyanus* nymphs was similar throughout the day in the two years. Interestingly, we also encountered a higher number of occupied fruit in the north side of the canopy at the end of the day (20:00) in 2010. However, this interaction was not significant in 2011, likely because population density was lower and temperature and light intensity lower (Table 2). The diurnal distribution of adult thrips has been widely studied (Tappan 1986, Sites et al. 1992, Cho et al. 2000, Ugine et al. 2006) but little is known about the diurnal distribution of immature thrips, the stage that generally causes damage. The few studies available do not report a clear pattern for *Frankliniella* on tomato (Salguero-Navas et al. 1991), cucumber (Kiers et al. 2000) and garden impatiens (Ugine et al. 2006) or for *Trips tabaci* Lindeman in onions (Mo et al. 2008). Only Tappan (1986) reported a peak of *F. fusca* immatures between 07:00 and 08:00 hours in peanuts.

In the context of developing a sampling plan or improving the targeting or timing of insecticidal spray applications, the distribution of *P. kellyanus* nymphs and damage in citrus indicates that sampling protocols and pesticide applications will need to include the upper half of the canopy. Navarro-Campos et al. (2012a) proposed a binomial sampling protocol based on the presence of immature thrips in fruit smaller than 4 cm in diameter. These authors recommend sampling 310 fruit, but they also suggest that this large number may be reduced at higher thrip population densities (Navarro-Campos et al. 2012a). Considering our results, this protocol may be reduced if growers sample the upper part of the canopy where thrips tend to aggregate. On the other hand, our study suggests that the effect of cardinal direction and time of the day are not so important when developing a sampling protocol and spraying insecticides. The effect of both factors on the percentage of occupied fruit did not follow the same pattern in two consecutive years within the same citrus orchard, indicating the variability of these factors.

Our results showed that fruit were occupied by nymphs of *P. kellyanus*, which are used to sample damaging populations, for approximately one day. If we consider that *P. kellyanus* remains in the nymph stage for more than four days at temperatures between 25 and 30 °C (Varikou et al. 2009), this short occupancy may be due to: i) the sampling protocol, which can bother the nymphs and modify their behavior; or ii) the mobility of the nymphs between fruits or between fruits and other plant organs. During this and other assays in the field, we have observed nymphs moving between fruits that are touching each other and on twigs and leaves close to fruits. Kirk (1985a, 1985b) reported that thrips nymphs move within the plant and these movements result in differences in their distribution within only a few hours. The mobility of *P. kellyanus* nymphs between fruits may explain, at least partially, the differences encountered between the low percentage of occupied fruit and the high percentage of damage by Navarro-Campos et al. (2013), as well as in our study.

To our knowledge, the effect of thrips on premature fruit abscission had not been tackled. Our results demonstrate that *P. kellyanus* nymphs induce the abscission of navel fruitlets. Other thrips, *Frankliniella bispinosa* (Morgan) and *F. kelliae* Sakimura, can cause premature flower abscission in navel and 'Valencia' oranges as a result of adult and larval feeding during bloom (Childers and Achor 1991). Similarly, the citrus rust mite *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae) can promote premature fruit abscission when feeding on the fruitlet surface (Allen 1978). Fruit growth is supported by the availability of nutrients, mostly mineral elements, carbohydrates and water. When carbohydrate supply for the fruit load is insufficient or when environmental conditions become adverse, new hormonal signals are generated to trigger temporal protection mechanisms, sometimes as drastic as fruit abscission (Iglesias et al. 2007; Gomez-Cadenas et al. 2000). The lack of carbohydrates in fruit produces the accumulation of ethylene in the peduncle, and consequently, fruit abscission occurs (Agustí 2000). Whether the feeding habits of *P. kellyanus* nymphs generate the accumulation of ethylene in the peduncle is unknown, but this premature abscission could be advantageous because it promotes the abscission of damaged fruit instead of healthy fruit. Further and more detailed research is necessary to determine how *P. kellyanus* might promote fruit abscission and also to test whether this premature abscission is economically advantageous for growers.

Acknowledgements

The authors thank Helga Montón and Pablo Bru from IVIA for technical assistance with experiments. This work was partially funded by the Spanish Ministry of Science and Innovation (projects: AGL2008-05287-C04/AGR and AGL2011-30538-C03-02) and the Conselleria d'Agricultura, Pesca i Alimentació de la Generalitat Valenciana. LP was the recipient of a grant from GV, and AT was the recipient of a postdoctoral fellowship from the MCINN (Juan de la Cierva program).

CAPÍTULO 5

Pezothrips kellyanus (Bagnall) (Thysanoptera: Thripidae) nymphs on orange fruit: importance of the second generation for its management

Pezothrips kellyanus (Bagnall) (Thysanoptera: Thripidae) nymphs on orange fruit: importance of the second generation for its management

Planes L, Catalán J, Jacas JA, Urbaneja A and Tena A. *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) nymphs on orange fruit: importance of the second generation for its management. *Florida Entomologist*,*98*(3), 848-855.

Abstract

Kelly's citrus thrips *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) is a new pest of oranges in New Zealand, southern Australia and the Mediterranean basin. The nymphs of this thrips can damage the fruit from petal fall up to six weeks later. Because there is a lack of information on its management, the aims of this study were to determine the number of generations occurring on the fruit and the efficacy of three insecticides (chlorpyrifos, spinosad and spirotetramat) to control this pest. Chlorpyrifos and spinosad displayed a high efficacy against *P. kellyanus* nymphs and reduced significantly the percentage of damaged fruit when a sole generation of *P. kellyanus* attacked the fruit. However, their activity did not prevent a subsequent generation of *P. kellyanus*. Interestingly, the percentage of damaged fruit increased, when this generation was detected. Spirotetramat did not display a knockdown effect and its efficacy was lower than that of chlorpyrifos and spinosad. Similar to these insecticides, spirotetramat did not prevent the attack of a second generation when it occurred. Additionally, we analyzed the side effects of these treatments on predatory mites. Spinosad and spirotetramat negatively affected these beneficial species.

Keywords: citrus, IPM, chlorpyriphos, spinosad, spirotetatramat, side-effects, predatory mites.

5.1 Introduction

Kelly's citrus thrips *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) is a new pest of citrus (Stevens et al. 1998; Webster et al. 2006; Vassiliou 2007; Navarro et al. 2008a). It became a pest in New Zealand (Blank and Gill 1997) and southern Australia (Mound and Jackman 1998) during the nineties. In Mediterranean basin, the first damage caused by *P. kellyanus* was recorded a few years later and nowadays it is considered a pest in Greece (Varikou et al. 2010), Cyprus (Vassiliou 2007), Sicily (Italy) (Marullo 1998; Conti et al. 2003) and Spain (Navarro et al. 2012a). *Pezothrips kellyanus* nymphs feed on the surface of citrus young fruits, for 5-6 weeks starting at petal fall (Navarro-Campos et al. 2013). This feeding habit causes patches or rings of scarred tissue around the fruit apex that enlarge as the fruit grows. This damage is particularly severe on navel oranges, lemons (Conti et al. 2003) and grapefruits (Mound and Jackman 1998; Baker et al. 2002; Vassiliou 2007; 2010). Although it does not affect the internal quality of the fruit, this damage leads to economic losses due to reduced market value of the affected fruits. The percentage of citrus fruits with a complete ring scar may reach 70% (Varikou 2002; Vassiliou 2010).

Despite the worldwide distribution and economic importance of *P. kellyanus*, its biological control is still under development (Baker 2011; Navarro-Campos et al. 2012b). Therefore, chemical control is nowadays the only practical alternative for growers. However, its implementation, results, and side effects are poorly known. First of all, the number of treatments necessary to reduce thrips populations is unclear. From one to three insecticide applications have been sprayed independently of their mode of action (Conti et al. 2004; Vassiliou 2007). Second, the efficacy of insecticides on *P. kellyanus* nymphs, those that produce the damage, has never been determined. The efficacy of the treatments has been determined based on fruit damage but whether these applications reduce either first or second thrips generations or both remains unknown. Third, the side effects of these treatments on the natural enemies of other important citrus pest have not been studied. Importantly, insecticides are sprayed in spring, when the populations of key natural enemies are increasing after winter (Martínez-Ferrer 2007; Tena et al. 2008; Sorribas et al. 2010; Urbaneja et al. 2008; Urbaneja et al. 2009). These natural enemies are responsible of the excellent biological control of many occasional and secondary citrus pests on orange cultivars in Spain (Jacas and Urbaneja 2009). Finally, *P. kellyanus* may develop resistance to insecticides if its chemical control relies only on organophosphates (Baker et al. 2004). Therefore, it is important to determine the efficacy against *P. kellyanus* of insecticides with different modes of action. Chlorpyrifos, an organophosphate insecticide, is one of the most-widely used active substances for pest control in citrus against hemipterans (scales and aphids) and thrips (Morse and Grafton-Cardwell 2012a; Navarro-Campos et al. 2012b; Planes et al. 2013). It is used against the later because of its fast-acting effect. However, its persistence against more than one generation of *P. kellyanus* is unknown. Spinosad is a mixture of tetracyclic-macrolide compounds, which has been identified as potential candidate for Integrated Pest Management (IPM) programs in citrus because of its fast action (insects dying of exhaustion within 1-2 days) and its low persistence (Thompson et al. 2000; Cisneros et al. 2002). Its residues on the leaf surface are degraded by sunlight in a few days (Salgado et al. 1998). For these characteristics, spinosad is recommended in citrus against *Scirtothrips citri* (Moulton) in California (Immaraju et al. 1989; Khan and Morse 2006; Morse and Grafton-Cardwell 2012b). Spirotetramat is a new systemic and persistent foliar insecticide. It is a tetramic acid derivative with a novel mode of action that interferes with lipid biosynthesis, leading to the death of immature stages of the target insect two to ten days after application (IRAC 2014). Spirotetramat is active against a wide spectrum of sucking insects, including aphids, scales (soft and armored), mealybugs, whiteflies, psyllids and selected thrips species (Grafton-Cardwell et al. 2007). Therefore, it could be used against *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) and *P. kellyanus* with a sole application at the end of spring. Moreover, its longer persistence could make it active against a possible second generation of *P. kellyanus*.

In this study, we determined: i) the efficacy of three insecticides with different modes of action (chlorpyrifos, spinosad and spirotetramat) against nymphs and adults of *P. kellyanus* in the field; ii) their persistence against subsequent generations of this thrips and iii) their efficacy to decrease the percentage of damaged fruit. These results would allow us to make an educated recommendation about the number of treatments necessary when these insecticides are used. Finally, iv) we also determined the side effects of these treatments on phytoseiid mite predators, one of the key group of natural enemies in citrus.

5.2 Material and Methods

5.2.1 Insecticides

The insecticides used in these assays were chlorpyrifos, spinosad and spirotetramat (Table 1). Following the recommendations of IPM for citrus (Urbaneja et al. 2015), insecticides were applied in the morning, when conditions were calm. The concentrations of the commercial products tested in these assays were the maximum authorized in citrus in Spain. For spirotetramat the concentrations used were recommended by the technical department of Bayer Crop Science (Valencia, Spain). Insecticides were applied when the percentage of occupied fruits was over the economic injury level set at 7% occupied fruits (Navarro et al. 2012a). For this purpose, orchards were sampled weekly for 5-6 weeks starting at petal fall.

Table 5. 1. Insecticides used in the assays.

Active Ingredient	AI g/l	Trade name	Company	Concentration (ml/hl)
Chlorpyrifos 48 % [EC] w/v	480	Dursban-48	Syngenta Agro, S.A.	200
Spinosad 48 % [EC] w/v	480	Spintor 480sc	Dow Agrosciences Iberica, S.A.	25
Spirotetramat 15 % [EC] w/v	150	Movento 150 OD	Bayer Cropscience, S.L.	50

5.2.2 Field assays

5.2.2.1 Alzira orchard

This assay was conducted in a 16-years-old navel orange ‘Lane-late’ orchard [*Citrus sinensis* Blanco var. Navel Lane-Late grafted on Citrange

'Carrizo' (*Citrus sinensis* L. Osbeck × *Poncirus trifoliata* Blanco)] located near the town of Alzira (UTM: X = 725190.34; Y = 4339126.26) (Valencia, Spain) in 2010. The orchard had 1.4 ha and the planting pattern was 6 x 5 m. It was drip irrigated and the naturally-occurring cover crop was mowed annually at the beginning of spring. The population density of nymphs was over the economic threshold at petal fall (May 25). This day, 35 trees were sampled and selected according to their similar infestation level by *P. kellyanus* nymphs (15-25% occupied fruits). The day after, insecticides were applied with a hand-gun, using outside coverage with a volume of about 4.5 l per tree (aprox. 1,500 l/ha). Ten, eight and eight trees (replicates) were sprayed with chlorpyrifos, spinosad and spirotetramat, respectively, and the remaining nine trees were not treated and considered as control. To avoid possible interferences, the eight trees surrounding every treated tree received the same treatment.

To monitor thrips populations and determine the efficacy of the insecticides, we sampled 32 fruits (eight per orientation) per tree one day prior to the spray, two days later and, then, weekly until the end of the study. On each fruit, we determined the presence of *P. kellyanus* nymphs. Insecticide efficacy was calculated using Abbott's formula (1925). The percentage of damaged fruit was determined on November 22 in the same trees. We sampled 40 fruits per tree. We differenced between slightly and severely damaged fruits. We considered a severely damaged fruits those that had complete ring-like scars and slightly damaged those with uncompleted ring-like scars.

5.2.2.2 Tavernes orchard

This assay was conducted in a 10-years-old navel orange 'Lane-late' orchard [*Citrus sinensis* Blanco var. Navel Lane-Late grafted on Cleopatra mandarin (*Citrus reshi* Hort. ex. Tan.)] located near the town of Tavernes de la Valldigna (UTM: X = 739997.68; Y = 4328225.46) (Valencia, Spain) in 2010. The orchard had 3.5 ha and the planting pattern was 6 x 4 m. It was drip irrigated and the naturally occurring cover crop was mowed annually at the beginning of spring. The experimental design was a randomized block with four replicates of four treatments. Each replicate contained three rows of 16-30 trees. The population density of nymphs exceeded the economic threshold two weeks after petal fall (May 31). This day, ten trees from each central row were labeled and sampled. One day later, 1,500 l/ha were applied with an air blast sprayer at 30 atm of pressure (Fede mod. Select dynamic; Fede S. L.; Cheste, Spain) to achieve outside tree coverage as is normal for citrus aphids-thrips treatments (Chueca et al., 2009). To follow thrips populations and determine the efficacy of the insecticides, we sampled 32 fruits per tree (eight per orientation) the day prior to the spray, two days later and then weekly until the end of the study. We determined the presence of *P. kellyanus* nymphs on each fruit. On November 23 the percentage of damaged fruit was determined as above.

To determine the population trends of *P. kellyanus* adults and natural enemies of citrus pests under the different insecticide treatments, a portable, engine-powered, suction device was used to collect all arthropods (Tena et al. 2008). The device was constructed by modifying a commercial vacuum-blower (Husqvarna Zenoah Co., model HBZ2601, Japan) adapted to collect insects from the foliage. We modified it by adding a cylindrical plastic pipe 50-cm long with a 30-cm diameter opening. The sampling was standardized by placing the opening of the cylindrical pipe a total of four times, during 5 to 8 seconds, on the foliage of ten citrus trees per date and tree (40 times in total). We sampled ten trees from the central row in each replicate (four replicates per treatment). The material collected was bagged and transported to the laboratory, where it was held at −20 °C to kill all insects. Adult thrips and natural enemies were counted and identified up to genus/species level under a binocular microscope. Insecticide efficacy on *P. kellyanus* adults was calculated using Abbott's formula (1925).

We also determined the side effects of the selected insecticides (Table 1) on phytoseiids. We counted the number of live phytoseiids on the underside of five interior and mature leaves per tree. Leaves were randomly selected in the canopies of the same trees sampled for *P. kellyanus*. The mean number of phytoseiids per leaf was determined for each block and treatment on each date sampled. Cumulative phytoseiids-days per leaf was calculated as an index of phytoseiid population for each replicate as:

$$\sum I_t((x_i+x_j)/2)$$

Where $\sum$ is summation over all sampling dates from the first evaluated day, on May 31, to the last one, on July 13, I_t is the interval between two successive sampling dates, and x_i and x_j are phytoseiid densities on those dates (Hardman et al. 2006; Kahn and Morse 2006).

5.2.3 Data analysis

Datasets were first tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Cochran's tests respectively, and transformed (angular transformation for percentage data) if needed. Subsequently, one-way ANOVA followed by Tukey hoc tests for multiple comparisons inside the different application time sub datasets were carried out.

5.3 Results

5.3.1 Efficacy against *Pezothrips kellyanus* nymphs

The percentage of fruits occupied by *P. kellyanus* nymphs exceeded the economic thresholds (7%) at petal fall in the orchard of Alzira and one week after the petal fall in Tavernes. This percentage was similar in all the treatments in both orchards (Table 2).

In Alzira, the percentage of occupied fruits was significantly higher in the control trees than in the treated trees two days after the treatments (Table 2). The efficacy of spinosad and chlorpyrifos, respectively, was significantly higher than that of spirotetramat (Table 2). Seven and 14 days after the treatments, the percentage of occupied fruits was low and there were no significant differences among treatments; therefore, efficacy could not be calculated. This percentage did not reach the economic threshold throughout the study.

In Tavernes, the percentage of occupied fruits was significantly higher in control and spirotetramat plots than in spinosad and chlorpyrifos plots two and seven days after the treatment (Table 2). The efficacy of spinosad and chlorpyrifos was high and there were no significant differences between them both days. Fourteen days after the treatment, the percentage of occupied fruits decreased and there were no significant differences among treatments. Twenty-one days after the treatments, the percentage of occupied fruits increased again and remained close to the economic thresholds for the following weeks in all the treatments. On July 8, the orchard was treated with chlorpyrifos and *P. kellyanus* populations decreased.

Table 5. 2. Occupancy (mean % ± SE) and efficacy (when occupancy was significantly different to control) of fruits by *Pezothrips kellyanus* in two orchards located in Alzira (A) and Tavernes (B) throughout the damaging period.

Orchard	Treatments	Day -1	Day 2		Day 7		Day 14	Day 21	Day 28
		% occupancy	% occupancy	% efficacy	% occupancy	% efficacy	% occupancy	% occupancy	% occupancy
Alzira	Control	20.00 ± 1.50	19.72 ± 2.45a		1.61 ± 0.56 a		2.22 ± 0.9	2.40 ± 0.33	1.61 ± 0.56
	Chlorpyrifos	22.25 ± 1.77	0.75 ± 0.38c	96.20 ± 1.9a	1.70 ± 0.39 a		2.75 ± 0.95	2.26 ± 0.36	1.70 ± 0.39
	Spinosad	18.13 ± 2.25	0.0 ± 0.0c	98.4 ± 1.58a	1.62 ± 0.34 a		0.31 ± 0.31	1.54 ± 0.26	1.62 ± 0.34
	Spirotetramat	20.63 ± 1.75	9.06 ± 1.70b	54.05 ± 8.6b	1.41 ± 0.31 a		1.56 ± 0.66	1.67 ± 0.32	1.41 ± 0.31
		$F_{3,34} = 0.88$ $P = 3.34$	$F_{3,34} = 38.12$ $P < 0.001$	$F_{2,25} = 27.39$ $P < 0.001$	$F_{3,34} = 0.09$ $P = 0.96$		$F_{3,34} = 1.79$ $P = 0.17$	$F_{3,34} = 1.76$ $P = 0.21$	$F_{3,34} = 0.09$ $P = 0.96$
Tavernes	Control	15.00 ± 1.15	10.16 ± 0.53a		2.44 ± 0.67a		2.03 ± 0.7	5.46 ± 1.47	6.09 ± 0.82
	Chlorpyrifos	13.44 ± 1.81	0.55 ± 0.15b	94.62 ± 1.47	0.63 ± 0.13b	74.19 ± 5.17	1.09 ± 0.53	5.46 ± 1.13	8.59 ± 0.86
	Spinosad	14.53 ± 3.04	1.02 ± 0.45b	90.00 ± 4.42	0.40 ± 0.24b	83.26 ± 9.88	0.96 ± 0.45	5.49 ± 1.48	7.69 ± 1.42
	Spirotetramat	14.90 ± 0.77	8.38 ± 3.00a		1.64 ± 0.34ab		1.44 ± 1.03	7.53 ± 3.02	5.61 ± 1.65
		$F_{3,15} = 0.14$ $P = 0.94$	$F_{3,15} = 10.15$ $P = 0.001$	$F_{1,7} = 0.18$ $P = 0.68$	$F_{3,15} = 5.51$ $P = 0.01$	$F_{1,7} = 1.40$ $P = 0.28$	$F_{3,15} = 0.43$ $P = 0.73$	$F_{3,15} = 0.25$ $P = 0.86$	$F_{3,15} = 1.11$ $P = 0.38$

5.3.2 Efficacy against *Pezothrips kellyanus* adults

Out of the 2,275 adult thrips collected with the vacuum device in Tavernes, 1951 (85.8%) were *P. kellyanus*. The number of *P. kellyanus* adults captured one day before the treatments was similar among treatments ($F_{3,\ 15} = 1.87$; $P = 0.19$) (Figure 5. 1). However, two days after the treatments, the number of adults increased and became significantly higher in control plots and in plots treated with spirotetramat ($F_{3,\ 15} = 8.59$; $P = 0.002$). The efficacy displayed by spinosad (89.4 ± 4.1 %) and chlorpyrifos (86.5 ± 4.4 %) was high and there were no significant differences between them ($F_{1,\ 7} = 0.22$; $P = 0.65$). Seven days after the treatments, the number of captured adults remained significantly lower than the control only in the plots treated with spinosad ($F_{3,\ 15} = 5.33$; $P = 0.015$). Fourteen days after the treatment, the number of captured adults decreased in the control plots and was the same in all treated plots ($F_{3,\ 15} = 0.68$; $P = 0.58$).

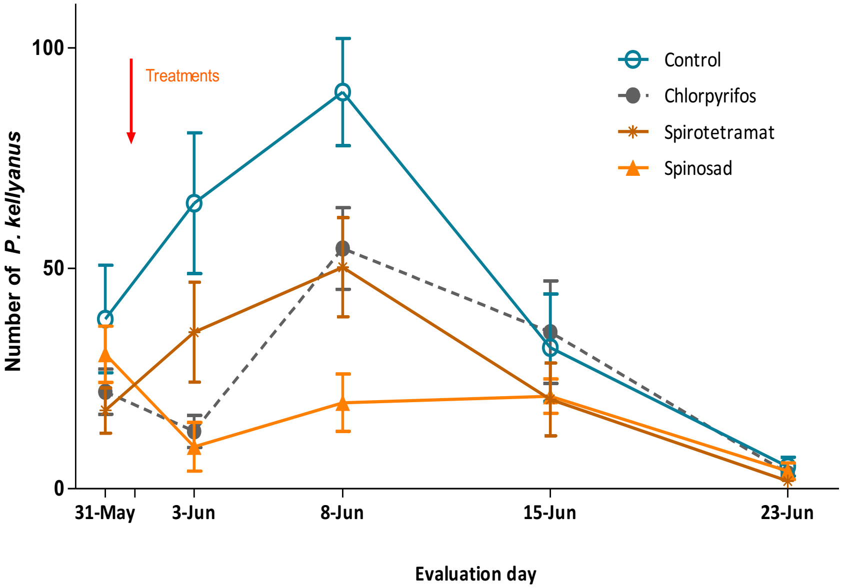


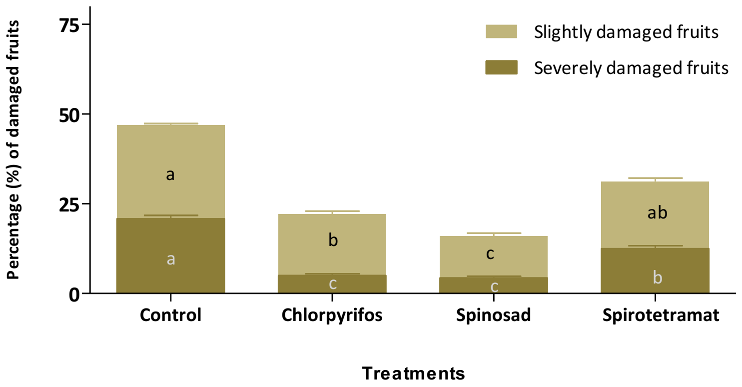
Figure 5. 1. Number of *Pezothrips kellyanus* adults collected with a vacuum device in a navel orchard located in Tavernes (mean ± SE). Trees were treated with chlorpyrifos, spinosad or spirotetramat.

5.3.3 Damage

In Alzira, the percentage of severely damaged fruits was significantly lower in the treated trees than in control trees (Fig. 2A) and it was significantly lower in trees treated with chlorpyrifos and spinosad than with spirotetramat ($F_{3,\ 34} = 13.85$; $P < 0.001$). The efficacy displayed by chlorpyrifos and spinosad was significantly higher than spirotetramat ($F_{2,\ 25} = 5.53$; $P = 0.01$). The percentage of slightly damaged fruits was significantly lower for the trees treated with chlorpyrifos and spinosad than control and spirotetramat trees ($F_{3,\ 34} = 5.72$; $P = 0.003$). There were no significant differences between the efficacy displayed by chlorpyrifos and spinosad ($F_{1,\ 16} = 1.81$; $P = 0.22$).

In Tavernes, however, the percentages of slightly and severely damaged fruits were high and there were no significant differences among the three treatments and the control (slightly damaged: $F_{3,\ 15} = 0.33$; $P = 0.09$; severely damaged: $F_{3,\ 15} = 0.53$; $P = 0.67$) (Fig. 2B).

a)



b)

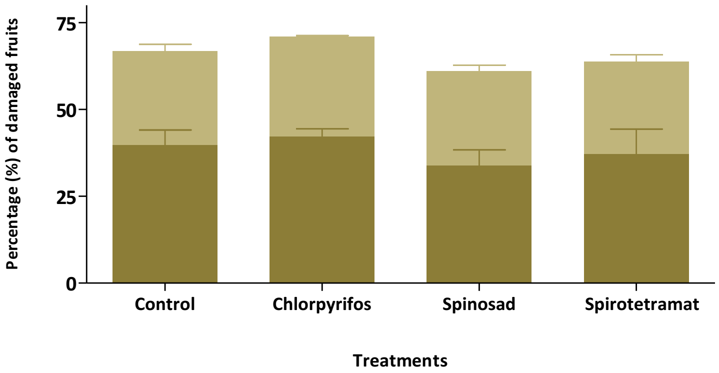


Figure 5. 2. Percentage (mean ± SE) of fruits slightly and severely damaged by *Pezothrips kellyanus* nymphs in every insecticide plot in orchard of Alzira (a) and Tavernes (b). Trees were treated with chlorpyrifos, spinosad or spirotetramat. Different letters indicate significant differences ($P < 0.05$) between treatments.

5.3.4 Side effects

The number of phytoseiids per leaf was similar in all plots the day previous to the treatments in Tavernes (Table 5.3). Their densities did not differ significantly the following days. However, the accumulated phytoseiid-day values, used as an overall summary statistic, were significantly lower in the plots treated with spinosad and spirotetramat than in those untreated or treated with chlorpyrifos.

Table 5. 3. Side effects of insecticides used against *Pezothrips kellyanus* on phytoseiids population. Number of phytoseiids and cumulative phytoseiids-days per leaf for each treatment.

Treatment	Mean number of phytoseiids per leaf ± SE								Cumulative phytoseiids-day
	Day -1	Day 2	Day 7	Day 14	Day 22	Day 28	Day 35	Day 42	
Control	0.83 ± 0.48 a	0.78 ± 0.32 a	1.99 ± 0.24 ab	1.45 ± 0.50 a	1.61 ± 0.56 a	2.40 ± 0.33 a	0.91 ± 0.09 ab	0.81 ± 0.14 a	63.12 ± 6.32 a
Chlorpyrifos	0.97 ± 0,36 a	0.65 ± 0.04 a	1.44 ± 0.19 ab	1.47 ± 0.24 a	1.70 ± 0.39 a	2.26 ± 0.36 a	1.08 ± 0.19 a	0.57 ± 0.11 a	59.76 ± 3.20 a
Spinosad	1.10 ± 0.40 a	0.35 ± 0.03 a	1.29 ± 0.19 b	1.08 ± 0.39 a	1.62 ± 0.34 a	1.54 ± 0.26 a	0.54 ± 0.13 b	0.56 ± 0.13 a	45.92 ± 2.10 b
Spirotetramat	0.70 ± 0.27 a	0.50 ± 0.09 a	1.31 ± 0.21 b	1.05 ± 0.27 a	1.41 ± 0.31 a	1.67 ± 0.32 a	0.67 ± 0.12 ab	0.61 ± 0.23 a	46.34 ± 3.04 b
	$F_{3,\ 15} = 0.20$ $P = 0.89$	$F_{3,\ 15} = 1.18$ $P = 0.35$	$F_{3,\ 15} = 2.53$ $P = 0.17$	$F_{3,\ 15} = 0.39$ $P = 0.76$	$F_{3,\ 15} = 0.09$ $P = 0.96$	$F_{3,\ 15} = 1.76$ $P = 0.21$	$F_{3,\ 15} = 2.93$ $P = 0.07$	$F_{3,\ 15} = 0.56$ $P = 0.65$	$F_{3,\ 15} = 5.01$ $P = 0.02$

We captured and identified 1,740 natural enemies with the vacuum device (Table 5.4; Figure 5.3). Hymenoptera parasitoids were the most abundant, a total of 927, followed by neuropteran predators (286) and arachnid predators (241). In general, the total number of natural enemies captured was higher in untreated plots (control) than in the treated plots in the following days. However, we could not determine the side effects of the insecticides on the main natural enemies of citrus, namely the parasitoids *A. melinus*, *Cales noacki* Howard (Aphelinidae), *Citrostichus phyllocnistoides* (Narayan) (Eulophidae) and *Metaphycus* spp. (Encyrtidae) and the predators of family Coccinelidae.

Table 5. 4. Side efects of insecticides used against Pezothrips kellyanus on natural enemies. Total number of insects captured with a Vacuum device per treatment and evaluation day.

Order/Family	Species/Genus	Previous				Two days after treatment				Seven days After treatment				Fourteen days after treatment				Twenty-one days after treatment				Total
		Cont	Chlor	Spin	Spiro	Cont	Chlor	Spin	Spiro	Cont	Chlor	Spin	Spiro	Cont	Chlor	Spin	Spiro	Cont	Chlor	Spin	Spiro	
Araneae		21	20	19	20	13	7	5	7	10	6	4	3	8	4	8	8	36	6	21	15	241
Coleoptera	*Scymnus*	0	1	1	1	0	1	0	1	3	2	3	2	6	11	11	8	18	10	7	16	102
Diptera																						
Cecidomidae	*Aphidoletes*	2	1	1	4	0	1	1	0	4	2	0	0	0	3	3	0	11	3	0	1	37
Hybotidae	*Platypalpus*	0	1	1	3	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	9
Heteroptera																						
Miridae	*Campyloneura virgula*	11	4	13	10	14	5	9	11	16	4	6	18	3	5	4	3	0	0	0	2	138
Hymenoptera																						
Aphelinidae	*Aphytis*	6	4	2	4	3	3	2	1	2	0	0	0	0	1	0	1	12	1	5	3	50
	Cales noacki	2	2	5	10	5	2	0	0	5	0	1	1	0	0	0	0	10	4	1	4	52
	Aphelinus	1	4	1	2	4	4	1	7	3	4	0	3	0	0	1	0	4	4	1	13	57
	Others	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	2
Braconidae	*Aphidiinae*	10	10	16	15	15	5	7	5	26	9	5	16	0	0	2	3	0	0	0	0	144
	Alysiinae	8	12	4	7	2	0	4	6	10	7	4	7	1	2	2	4	3	6	6	8	103
	Others	3	4	3	3	3	1	1	4	1	3	6	0	2	1	1	1	3	0	2	3	45
Ceraphronoidea		12	7	19	12	10	5	3	5	11	10	10	5	6	8	14	5	21	16	23	23	225
Cynipoidea		0	2	4	4	3	3	3	7	8	6	5	4	0	1	4	1	2	3	0	0	60
Encyrtidae	*Metaphycus*	2	2	4	1	1	2	0	1	3	2	0	0	3	5	1	3	5	5	5	1	46
	Others	1	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	10	10	10	8	43
Platygastroidea		1	6	0	5	5	2	0	0	3	3	3	1	2	5	4	1	9	7	10	5	72
Pteromalidae		5	4	2	1	0	0	1	0	1	0	2	0	0	0	0	1	3	7	1	0	28
Neuroptera																						
Coniopterigydae	*Conwentzia psociformis*	3	2	4	4	1	3	0	2	3	4	1	3	6	2	0	4	8	9	9	4	72
	Semidaelis aleyrodiformis	1	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	1	0	7
Chrysopidae	*Chrysoperla carnea*	1	2	2	3	5	1	0	0	5	6	1	6	7	13	4	4	48	57	26	16	207
Total		**90**	**89**	**101**	**109**	**84**	**45**	**37**	**57**	**117**	**69**	**52**	**71**	**48**	**61**	**60**	**47**	**204**	**149**	**128**	**122**	**1740**

5.4 Discussion

Our results, based on weekly monitoring *P. kellyanus* immature populations on fruit, indicated that a sole insecticide application of either chlorpyrifos or spinosad can suppress *P. kellyanus* nymphs when only one generation of *P. kellyanus* attack the fruit. This was the case of the assay in Alzira. The organophosphate, chlorpyrifos, and spinosad displayed a knockdown effect against *P. kellyanus* nymphs and, two days after the treatment, reduced the percentage of occupied fruits below economic thresholds. Both pesticides reached efficacies higher than 90%. Afterwards, thrips populations remained low in both treated and untreated trees. Thus, only one generation attacked the fruit in this assay. As consequence of this attack, the percentage of damaged fruit at harvest was lower than 25% in the trees treated with chlorpyrifos or spinosad whereas it reached almost 50% in untreated trees. Therefore, these insecticides are able to control *P. kellyanus* and its damage with a sole application when only one generation of this thrips attacks the fruit. Both pesticides had been previously tested against this pest with similar results (Benfatto et al. 2000; Purvis et al. 2002). Baker et al. (2004) considered spinosad as a potential candidate for IPM of *P. kellyanus* in Australia and Vassiliou (2007) identified chlorpyrifos as the most effective insecticide from fifteen tested in his study. The results of these two studies are based on the observation of damaged fruit at harvest but they did not monitor *P. kellyanus* populations neither before nor after the treatments.

By contrast, our assay in Tavernes showed that a sole application of chlorpyrifos or spinosad cannot suppress a second attack of *P. kellyanus*. As in Alzira, both insecticides reduced the percentage of occupied fruits under economic thresholds two days after the treatment. These percentages remained low for 21 days after the treatments, when a new generation of nymphs attacked the fruit again in all blocks. Thus, the persistence of chlorpyrifos and spinosad applied against the first generation was not enough to control the second one. Consequently, a second treatment would have been necessary to suppress it. Although the percentage of occupied fruits was three times lower than in the first generation and fruit size was higher (it measured 4.49 ± 0.09 cm on July 13), this second application seems necessary because the percentage of damaged fruit was very high (above 50%) in this assay. Importantly, all insecticides applied against the first generation were able to reduce the percentage of damaged fruit when compared with the control. Vassiliou et al. (2007) sprayed twice against *P. kellyanus*, but the percentage of damaged fruit was approximately 70%. Therefore, a second application does not guarantee a reduction of damaged fruits. In their assay, they did not monitor *P. kellyanus* populations and it is not known whether the application timing was correct. Consequently, this second application can be only recommended when thrips populations are monitored. Finally, if a second treatment is necessary, the insecticides used should be different from those used against the first generation to avoid resistances and to assure the effectiveness of the available pesticides.

Some populations of citrus thrips have developed resistance to pesticides (Morse and Brawner 1986, Immaraju et al. 1989). In Californian citrus, *S. citri* developed resistance to a long list of insecticides (Morse and Brawner 1986; Immaraju et al. 1989; Khan and Morse 1998). Baker et al. (2004) found that some *P. kellyanus* populations in Southern Australia had substantial levels of chlorpyrifos resistance. In Spain, chlorpyrifos has been widely used to control *A.*

aurantii and other armored scales during the last two decades. The high efficacy obtained with chlorpyrifos in our assays suggests that Spanish populations of *P. kellyanus* have not yet developed resistance to this insecticide. To avoid resistant populations, citrus growers should avoid applying chlorpyrifos against both generations of *P. kellyanus* or against *A. aurantii* and *P. kellyanus* within the same year. The most obvious way of delaying the development of resistance to insecticides is to use them only when required, relying whenever possible on other methods of control that are included into IPM programs to prevent crop damage (Morse and Brawner 1986). In Spain, growers spray twice during this period to protect the fruit from *P. kellyanus* scarring. The first treatment is generally applied at petal fall and the second one is usually applied 15 days later as a routine. However, insecticides should not be applied at petal fall as a habitual practice because the first generation may appear later, as occurred in Tavernes' assay. Furthermore, the existence of a second generation may vary among years and locations. In fact, we did not observe a second generation of nymphs in this orchard in 2012 (pers. observations). Consequently, a detailed monitoring is critical to determine the optimum spray timing, assure the efficacy of treatments and delay the appearance of resistant populations. In California, timing is considered vital to achieve adequate control of *S. citri* with a single application of a relatively short residual pesticide, so that destruction of beneficial organisms is minimized (Morse and Brawner 1986; Morse et al. 1988).

Spirotetramat has been recently registered against *A. aurantii*, *Panonychus citri* and thrips (Grafton-Cardwell et al. 2007; Grafton-Cardwell and Scott 2008; Morse and Grafton-Cardwell 2009; MAGRAMA 2014). It might be an interesting insecticide as it could control *A. aurantii* and *P. kellyanus* with a single application in spring.

In our study, spirotetramat showed an efficacy around 60 % and reduced both slight and severe scarring around 40 % relative to control in Alzira in 2009. However, its efficacy was lower than chlorpyrifos and spinosad and, more importantly, it did not display a knockdown effect. Likely, because its contact efficacy is rather limited (Nauen et al. 2008). Thus, its effect on *P. kellyanus* seems to be limited when compared to spinosad and chlorpyrifos. Moreover, despite of its systemic and translaminar activity (Nauen et al. 2008), it did not avoid the attack of the second generation in Tavernes.

To avoid the disruption of the excellent biological control of some important orange pests (Jacas and Urbaneja 2009) the insecticides selected against *P. kellyanus* should have relatively short residual effects, so their impact on beneficial organisms is minimized (Morse and Brawner 1986; Morse et al. 1988). This is especially important because *P. kellyanus* is treated at the end of spring when most natural enemy populations are increasing in Spanish citrus (Martínez-Ferrer 2003; Tena et al. 2008; Sorribas et al. 2010; Urbaneja et al. 2008; Urbaneja et al. 2009). Spinosad and spirotetramat decreased the number of cumulative phitoseiids-day. Spinosad also reduced the number of cumulative phytoseiids-day in a similar study carried out in California (Kahn and Morse 2006). Although spinosad and spirotetramat showed a low toxicity in our study, a more detailed study should clarify the side effect of spinosad on phytoseiids because it was highly effective against *P. kellyanus* and is therefore a candidate to be used within IPM programs. This is especially relevant if two treatments are necessary to control *P. kellyanus*. Apart from counting phytoseiids, we also collected beneficial insects with a vacuum device. However, we could not determine the side effects of the insecticides tested on the other two groups of natural enemies that are key to Spanish IPM programs in citrus, namely coccinellid predators and hymenopterous parasitoids (Urbaneja et al. 2008). Therefore, we would recommend determining the side effects on representative parasitoids and coccinelids of citrus IPM programs under laboratory conditions to ascertain its actual impact. Some of these studies have already demonstrated that chlorpyrifos results harmful for the parasitoid *A. melinus* (Vanaclocha et al. 2013; González-Zamora et al. 2013) and the coccinelid *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) (Planes et al. 2013).

In conclusion, our study shows that chlorpyrifos and spinosad display a knockdown effect and can control the first generation of *P. kellyanus* in citrus with a sole application. However, their persistence on adults is not enough to avoid a second generation when it occurs. Therefore, an additional application might be necessary in those cases where this second generation occurs. However, this second application, as the first one, is only justified when thrips populations are correctly monitored and result above action thresholds. Finally, as exposed herein, IPM programs on navel oranges in Spain is based on biological control of most of their pests. Consequently, the development of alternative control strategies to avoid the disruption of the established biological control is urgently needed.

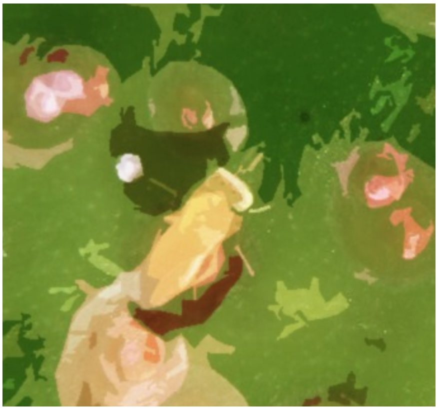
Acknowledgements

We are grateful to Bautista Domènech and Bernardo Villalba for allowing us to sample their orchards. We also thank P. Bru, H. Montón, C. Monzó, B. Sabater, E. Llácer, Khaled, F. Gómez-Marco (IVIA) for their technical assistance and J. Izquierdo (Bayer Crop Science, Spain) for its collaboration. L. Planes was a recipient of a fellowship from IVIA. A. Tena was a recipient of a postdoctoral fellowship from the MCINN

(Juan de la Cierva program). This research was partly funded by the Spanish Ministry of Science and Innovation (project: AGL2008-05287-C04/AGR) and by the Conselleria d'Agricultura, Pesca i Alimentació from Generalitat Valenciana.

CAPÍTULO 6

Side effects of pesticides used against *Pezothrips kellyanus* on natural enemies



6.1 Lethal and sublethal effects of spirotetramat on the mealybug destroyer, *Cryptolaemus montrouzieri*

Planes L, Catalán J, Tena A, Porcuna JL, Jacas JA, Izquierdo J and Urbaneja A. 2013. Lethal and sublethal effects of spirotetramat on the mealybug destroyer, *Cryptolaemus montrouzieri*. Journal Pest of Science 86:321-327.

Abstract

Spirotetramat is a new systemic insecticide listed in Group 23 of the IRAC mode-of-action classification scheme as an inhibitor of lipid biosynthesis. Side effects assessment on key natural enemies is necessary before incorporating a pesticide in IPM programs. Herein, lethal and sublethal side effects of spirotetramat on adults and larvae of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) were evaluated under laboratory conditions by topical application and by ingestion of treated individuals of *Planococcus citri* Risso (Hemiptera: Pseudococcidae). The lethal and sublethal effects of spirotetramat were compared to those of chlorpyriphos and pyriproxyfen, two insecticides commonly used in Spanish citrus. Spirotetramat resulted harmless: (1) when directly applied on larvae and adults of *C. montrouzieri*, since it did not affect survival, longevity, fecundity, egg hatching, and offspring survival. In contrast, chlorpyriphos was classified as moderately toxic for adults due to its effects on fecundity, egg hatching and offspring survival. Pyriproxyfen was classified as harmful for larvae due to the acute effect on pupal mortality. When larvae and adults of *C. montrouzieri* were fed with treated prey, spirotetramat was also classified as harmless. Adults of *C. montrouzieri* fed with pyriproxyfen-treated prey exhibited increased fecundity but no eggs hatched. Moreover, the larvae fed on pyriproxyfentreated prey did not reach the adult stage. The results of this study indicate that spirotetramat may be compatible with augmentative releases of *C. montrouzieri* in citrus.

Keywords: IPM, Side effects, Chlorpyriphos, Pyriproxyfen, *Planococcus citri*.

6.1.1 Introduction

Coccinellids (Coleoptera: Coccinellidae) are one of the most important groups of predators in biological control programs (Hagen et al. 1999) and usually play a key role in integrated pest management (IPM) programs in several agroecosystems, such as citrus in Spain (Jacas and Urbaneja 2010). Several important citrus pest are attacked by naturally occurring coccinellids (Michelena and Sanchis 1997; Alvis-Da´vila et al. 2002; Abad-Moyano et al. 2009; Urbaneja et al. 2015) and among them, the mealybug destroyer Cryptolaemus montrouzieri Mulsant is periodically introduced by means of augmentative releases against the citrus mealybug Planococcus citri (Risso) (Hemiptera: Pseudococcidae) (Martínez-Ferrer et al. 2003; Jacas and Urbaneja 2010). C. montrouzieri was introduced in a classical biological control program but failed to establish in Spanish citrus. Its current use involves adult releases during spring, when P. citri gravid females are present, at a dose of three to ten adults per tree repeated every 14–21 days (Jacas et al. 2006; Jacas and Urbaneja 2010).

This successful release strategy is used in other Mediterranean countries (Katsoyannos 1997), as well as in areas with a similar climate, such as Australia and California (UC 1991; Smith et al. 1997). C. montrouzieri is also considered an important predator in vineyards and ornamental plants in greenhouses (Daane et al. 2007; Mus¸tu et al. 2008).

One of the most popular tactics used for the conservation of natural enemies in Spanish citrus has been the use of pesticides with a reduced impact on beneficial arthropods by exploiting either their intrinsic or their ecological selectivity (Croft 1990; Urbaneja et al. 2015). For this reason, newly developed pesticides must be subjected before their registration process to the risk assessment toward some selected non-target organisms, such as predators and parasitoids (EC 2009).

A new systemic and persistent foliar insecticide is spirotetramat, a tetramic acid derivative with a novel mode-of-action that interferes with lipid biosynthesis. Spirotetramat is listed in Group 23 of the Insecticide Resistance Action Committee (IRAC 2012) together with spirodiclofen and spiromesifen. It causes the death of immature stages of the target insect from 2 to 10 days after application (Nauen et al. 2008). Spirotetramat is used against Aonidiella aurantii (Maskell) (Hemiptera: Diaspididae) and other sucking pests, such as aphids, scales (soft and armored), mealybugs, whiteflies, psyllids, and some thrips species in Californian citrus (Grafton-Cardwell et al. 2007; Grafton-Cardwell and Scott 2008). In 2011, the use of this pesticide was authorized in several European countries (i.e., United Kingdom, Belgium, and Switzerland) in different crops, such us brassicas and lettuce to control sucking pests (Bayer Crop Science 2012). Despite its use in California and its possible use in other citrus areas, the side effects of spirotetramat on coccinellids are still poorly known (Brück et al. 2009).

Therefore, the objective of this study is to assess the lethal and sublethal side effects of spirotetramat on the immature stages and adults of C. montrouzieri exposed to the pesticide through topical application or ingestion of treated prey (*P. citri*).

6.1.2 Materials and methods

6.1.2.1 Insecticides

The concentration of spirotetramat (Movento 150 OD®; 150 g of a.i. per liter) used in our assays was the maximum proposed by Bayer Crop Science (Valencia, Spain) for the Spanish register, 50 ml/hl. Distilled water was used as untreated control in all treatments. The juvenile hormone analogue pyriproxyfen (Juvinal®, Kenogard S.A.; 100 g of a.i. per liter) at 75 ml/hl, and the organophosphate chlorpyriphos (Dursban®, Dow Agrosciences Iberica S.A.; 480 g of a.i. per liter) at 200 ml/hl, two products commonly used against *P. citri* in Spain (MARM 2012), were used as treated controls. These two insecticides were selected as control treatments because of their known toxicity against different stages of *C. montrouzieri* (Boyero et al. 2005; Pascual-Ruiz and Urbaneja 2006). A Potter Spray Tower (Burkard Scientific Ltd®, Uxbridge, UK) was used to apply the insecticides (Figure 6.1.1).

In all the experiments (topical and ingestion), 2 ml of the corresponding product dilution were sprayed at 150 kPa resulting in a spray deposit of 1.5 mg/cm2 (Hassan 1998).

Figure 6.1. 1. Potter tower used to apply the insecticides.

6.1.2.2 Insects and experimental conditions

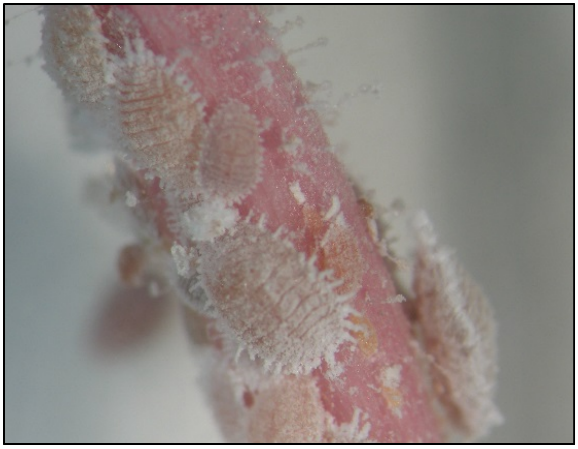


Figure 6.1. 2. Mealybug *P. citri* on potato sprouts.

Adults and larvae of *C. montrouzieri* used in this assay were obtained from the State Insectary of Valencia (Spain) where they were reared on the citrus mealybug *P. citri* on potato sprouts (Figure 6.1.2). Adults were kept in plastic vials where food, a mixture of honey and agar (50 % w/v), was smeared on the inner side of the lid. First instar larvae were collected from potato sprouts infested by *P. citri*. Prey was also obtained from potato sprouts which originated from the same insectary.

Environmental conditions in all experiments were 25 ± 2 °C, 60 ± 10 % RH and a photoperiod of 16:8 h (L:D).

6.1.2.3 Adult topical treatment

Ten-day-old *C. montrouzieri* adults were sprayed with spirotetramat, chlorpyriphos, or distilled water (control). Six couples per replicate were placed on a polyethylene mesh (220 - 331-μm mesh) to permit excess liquid run off and were sprayed in the Potter tower. Ten replicates were conducted for each treatment. Immediately after spraying, one couple per replicate was randomly selected to study their reproductive parameters (fecundity, egg hatching and immature stages survival of the progeny) and the remaining five couples were used to study adult mortality and longevity following the methods developed by Urbaneja et al. (2008).

To study adult mortality and longevity, treated couples were transferred into a Petri dish 150 mm in diameter. Mortality was recorded daily until day 40 after treatment.

Adults were fed a mixture of honey in agar (50 % w/v) smeared in a piece of plastic (2 × 3 cm) introduced into the experimental arena (Castañer 1992; Urbaneja et al. 2008) (Figure 6.1.3).

Figure 6.1. 3. Mixture of honey in agar smeared in a piece of plastic.

To study the reproductive parameters, couples were individually transferred with a fine brush to a plastic Petri dish 90 mm in diameter (Figure 6.1.4).

Figure 6.1. 4. Couples of *C. montrouzieri* individually transfered to study reproductive parametres**.**

The side of each Petri dish had 4 holes of 7 mm in diameter which were covered by gauze.

Couples were kept inside the cage for 10 days and fed with untreated prey. Prey was offered on a piece of paper (2 × 3 cm), which was used to collect mealybugs from infested sprouts. Prey was always offered in excess to guarantee both, food and oviposition substrate, for *C. montrouzieri* females (~10 *P. citri* nymphs of all instars and more than 150 eggs). These pieces of paper were replaced daily and checked under a binocular microscope to ascertain *C. montrouzieri* fecundity. The pieces of paper were transferred to another Petri dish 60 mm in diameter where egg hatching was measured daily (Figure 6.1.5).

Figure 6.1. 5. Egss of *C. montrouzieri* (white) and eggs of *P. citri* (orange).

Upon hatching, five randomly selected larvae per individual couple less than 24 h old were transferred to a new Petri dish measuring 60 mm in diameter. Larvae were fed ad libitum with untreated *P. citri* until they reached the adult stage (Figure 6.1.6 and 6.1.7). Immature survival was recorded.

Figure 6.1. 6. Larvae of *C. montrouzieri*.

Figure 6.1. 7. Pupae and adult of *C. montrouzieri*.

6.1.2.4 Larvae topical treatment

A group of 20 larvae of *C. montrouzieri* less than 24 h old (first instar; Figure 6.1.8) were treated with spirotetramat, pyriproxyfen or distilled water (control) in the Potter tower following the method described for adults. Five replicates were conducted per treatment.

Figure 6.1. 8. First instar larvae of *C. montrouzieri.*

Larvae were transferred to a 90 mm diameter Petri dish and fed untreated prey. Immature mortality was evaluated daily under a binocular microscope until the larvae reached the adult stage. Then ten adult couples per treatment were further monitored following the protocol used for adults to ascertain their reproductive parameters.

6.1.2.5 Ingestion of treated prey by adults and larvae

In this study, *C. montrouzieri* larvae and adults were exposed to pesticides through ingestion of treated prey rather than topical treatment. The design and replication of the study was the same as for the topical treatment of adults and larvae. *P. citri*-infested potato sprouts were directly sprayed (Figure 6.1.9) in the Potter Spray Tower with spirotetramat, pyriprixofen or distilled water (control) using the same method described in the ''Adult topical treatment'' section.

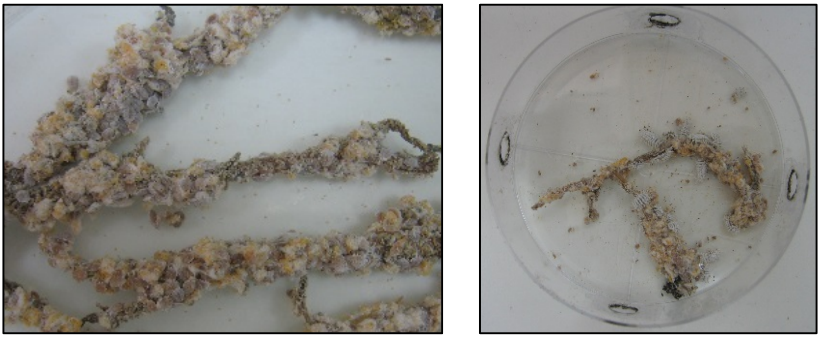


Figure 6.1. 9. *P. citri*-infested potato sprouts were directly sprayed in the Potter Spray Tower.

Only the parent generation was fed with treated prey (Figure 6.1.10). Descendants were fed with untreated *P. citri*.

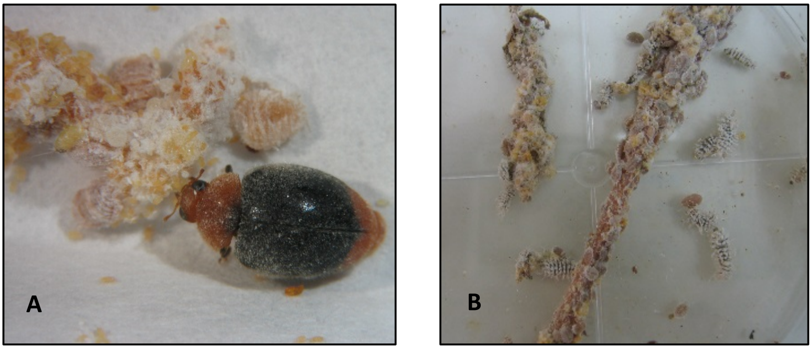


Figure 6.1. 10. Ingestion of treated prey *P. citri* by adults (A) and larvae (B) of *C. montrouzieri*.

6.1.2.6 Data analysis

Adult survival until 40 days after the treatments for both, topical and ingestion experiments were analyzed by Kaplan–Meier survival analysis. Within the Kaplan–Meier procedure, cumulative survival functions (combination of survival time and survival probability) were compared among treatments by Breslow test.

Other biological parameters (fecundity, egg hatching, and immature survival) were compared by ANOVA. The LSD test was used for mean separation at $P < 0.05$. When significant differences were found ($P < 0.05$), the corresponding reduction value (RV) was calculated using the Abbott formula (Abbott 1925). When no significant differences were found the RV was assigned as 1. Subsequently, the different RVs obtained for the same product in an experiment (Urbaneja et al. 2008) were combined as follows:

$E = 100 \times (1 - (RV_{\text{Adult survival}} \times RV_{\text{Fecundity}} \times RV_{\text{Egg hatching}} \times RV_{\text{Immature survival}})$

Each term represents the percentage reduction in relation to the control for the different parameters considered. The resulting value (E) was interpreted according to International Organization of Biological Control (IOBC) standards (Sterk et al. 1999), that include four categories (1) harmless; (2) slightly harmful, (3) moderately harmful and (4) harmful, which correspond to reductions below 30 %, between 31 and 79 %, between 80 and 99 % and higher than 99 %, respectively.

6.1.3 Results

6.1.3.1 Adult topical treatment

Breslow tests did not reveal significant differences among treatments for the survival of *C. montrouzieri* adults ($U = 0.11$; $P = 0.74$) (Table 1; Fig. 1). Fecundity, egg hatching and progeny immature stage survival were not affected by spirotetramat; however, these parameters were reduced in the case of chlorpyriphos (Table 6.1.1).

Table 6. 1. 1. Cumulate survival (%), fecundity (eggs/female during 10 days), egg hatching (%), and F1 survival (%) of *Cryptolaemus montrouzieri* adults exposed to different products and water by direct spray.

	Chlorpyrifos	Spirotetramat	Control	Statistics
Cumulative survival[a]	28.4 ± 0.1a [1.00]	28.6 ± 1.0a [1.00]	30.0 ± 0.1a	
Fecundity	64.4 ± 14.2b [0.60]	82.6 ± 10.6ab [1.00]	107.0 ± 6.1a	$F = 3.8$; df = 2, 29; $P = 0.0332$
Egg hatching	37.2 ± 9.9b [0.53]	62.3 ± 8.2a [1.00]	70.4 ± 5.1a	$F = 4.7$; df = 2, 29; $P = 0.018$
F1 immature stages survival	50.0 ± 9.8b [0.57]	86.6 ± 9.8a [1.00]	88.0 ± 3.8a	$F = 6.73$; df = 2, 29; $P = 0.0043$
***E* (%) (IOBC cat.)**	81.8 **(3)**	0.0 **(1)**		

Initial numbers of *C. montrouzieri* adults were 5 couples per replicate and 10 replicates were considered per treatment. Reduction factors for each parameter appear in brackets and the IOBC category (IOBC cat.) corresponding to the total reduction value (*E*) is shown in the last row

[a] Within a row, data followed by a different letter are significantly different ($P < 0.05$, LSD test)

6.1.3.2 Larvae topical treatment

Spirotetramat did not affect larval and pupal mortality when first instar larvae were directly sprayed (Table 6.1.2). Contrarily, pyriproxyfen significantly increased pupal mortality. Similarly, spirotetramat did not affect fecundity during the first 10 days of oviposition; however, the fecundity of those individuals treated with pyriproxyfen was nil. Furthermore, egg hatching and F_1 immature stage survival were not affected by spirotetramat.

Table 6. 1. 2. Larval mortality (%), pupal mortality (%), fecundity (eggs/female during 10 days), egg hatching (%), and F1 survival (%) of *Cryptolaemus montrouzieri* larvae exposed to different products and water by direct spray.

	Pyriproxyfen	Spirotetramat	Control	Statistics
Larval mortality[a]	6.0 ± 1.8a [1.00]	2.0 ± 1.2a [1.00]	4.8 ± 1.5a	*F* = 1.7; df = 2, 14; *P* = 0.23
Pupal mortality	89.9 ± 2.3a [0.11]	2.0 ± 1.2b [1.00]	4.8 ± 1.5b	*F* = 779,8; df = 2, 14; *P* < 0.0001
Fecundity	0.0 ± 0.0b [0.00]	149.2 ± 8.2a [1.00]	170.9 ± 10.92a	*F* = 139.2; df = 2, 29; *P* <0.0001
Egg hatching	–	86.6 ± 2.5a [1.00]	93.0 ± 1.5a	*F* = 4.71; df = 1, 19; *P* = 0.0436
F1 immature stages survival	–	60.0 ± 5.3a [1.00]	63.3 ± 3.2a	*F* = 0.29; df = 1, 19; *P* = 0.6
***E* (%) (IOBC cat.)**	100.0 **(4)**	0.0 **(1)**		

Initial numbers of *C. montrouzieri* larvae were 20 per replicate and 5 replicates were considered per treatment. Reduction factors for each parameter appear in brackets, and the IOBC category corresponding to the total reduction value (*E*) is shown in the last row

[a] Within a row, data followed by a different letter are significantly different (P < 0.05, LSD test)

6.1.3.3 Ingestion of treated prey by adults

The longevity of C. *montrouzieri* adults feeding on prey sprayed with spirotetramat, pyriproxyfen or distilled wáter was similar ($U = 1.66$; $P = 0.20$) (Table 6.1.3; Figure 6.1.11). Spirotetramat did not affect the fecundity of *C. montrouzieri* females during the first 10 days of oviposition, egg hatching, and survival of the emerged F_1 immature stage survival. By contrast, pyriproxyfen increased the fecundity of C. *montrouzieri*; however, these eggs did not hatch.

Table 6. 1. 3. Cumulate survival (%), fecundity (eggs/female during 10 days), egg hatching (%), and F1 survival (%) of *Cryptolaemus montrouzieri* adults exposed to different products by ingestion of treated and untreated prey (*Planococcus citri*).

	Pyriproxyfen	Spirotetramat	Control	Statistics
Cumulative survival[a]	33.1 ± 0.1a [1.00]	34.1 ± 0.1a [1.00]	32.1 ± 1.7a	
Fecundity	183.5 ± 18.3a [1.00]	95.5 ± 18.1b [1.00]	102.1 ± 21.3b	$F = 6.42$; df = 2, 29; $P = 0.0052$
Egg hatching	0.0 ± 0.0b [0.00]	79.6 ± 4.8a [1.00]	76.0 ± 2.1a	$F = 217.2$; df = 2, 29; $P < 0.0001$
F_1 immature stages survival	-	88.6 ± 3.3a [1.00]	88.6 ± 2.8a	$F = 0.0$; df=1, 19; $P = 1.0$
***E* (%) (IOBC cat.)**	100.0 **(4)**	0.0 **(1)**		

Initial numbers of *C. montrouzieri* larvae were 20 per replicate and 5 replicates were considered per treatment. Reduction factors for each parameter appear in brackets, and the IOBC category corresponding to the total reduction value (*E*) is shown in the last row

[a] Within a row, data followed by a different letter are significantly different ($P < 0.05$, LSD test)

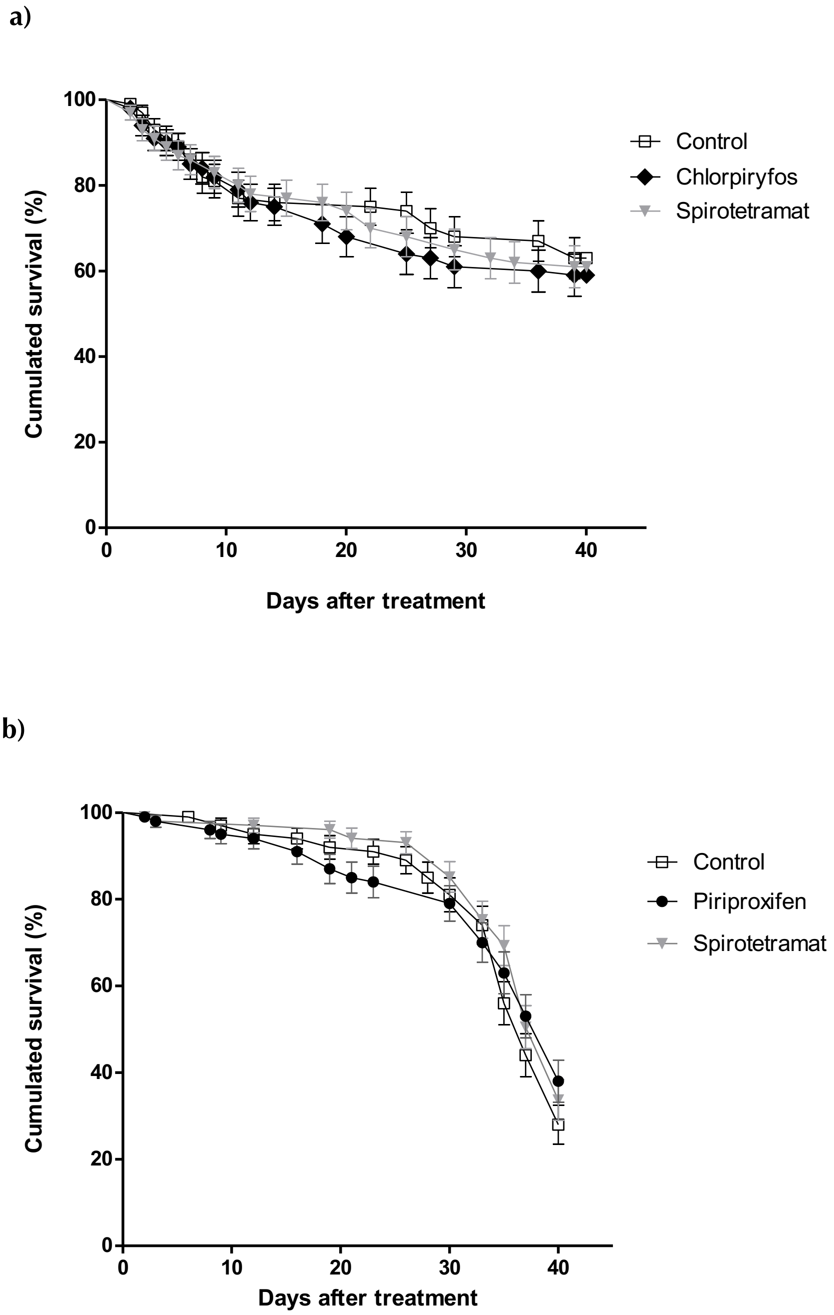


Figure 6.1. 11. Adult survival of *Cryptolaemus montrouzieri* when they were a) direct sprayed and b) fed on treated prey.

6.1.3.4 Ingestion of treated prey by larvae

Spirotetramat did not affect larval and pupal mortality of *C. montrouzieri* when larvae fed on treated preys (Table 6.1.4). Moreover, adult females obtained from these larvae exhibited a similar fecundity, egg hatching and offspring survival compared with those fed on untreated prey. However, all larvae fed on pyriproxyfen-treated prey died.

Table 6. 1. 4. Larval mortality, (%), pupal mortality (%), fecundity (eggs/female during 10 days), egg hatching (%), and F_1 survival (%) of *Cryptolaemus montrouzieri* larvae exposed to different products by ingestion of treated and untreated prey (*Planococcus citri*).

	Pyriproxyfen	Spirotetramat	Control	Statistics
Larval mortality^a	78.0 ± 0.4a [0.23]	2.0 ± 1.2b [1.00]	3.0 ± 0.3b	F = 177.5; df = 2, 14; P < 0.0001
Pupae mortality	100.0 ± 0.0a [0.00]	17.0 ± 4.8b [1.00]	5.0 ± 3.1b	F = 466.6; df = 2, 14; P < 0.0001
Fecundity	–	134.7± 10.6a [1.00]	149.4 ± 13.7a	F = 0.72; df = 1, 19; P = 0.41
Egg hatching	–	88.3 ± 3.3a [1.00]	92.0 ± 3.0a	F = 0.67; df = 1, 19; P = 0.42
F_1 immature stages survival	–	57.3 ± 6.9a [1.00]	72.0 ± 2.6a	F = 3.89; df = 1, 19; P = 0.064
E (%) (IOBC cat)^b	100.0 **(4)**	0.0 **(1)**		

Initial numbers of *C. montrouzieri* larvae were 20 per replicate and 5 replicates were considered per treatment. Reduction factors for each parameter appear in brackets, and the IOBC category corresponding to the total reduction value (*E*) is shown in the last row

[a] Within a row, data followed by a different letter are significantly different ($P < 0.05$, LSD test)

6.1.4 Discussion

Our results show that spirotetramat could be compatible with augmentative releases of the coccinellid *C. montrouzieri*. Following the guidelines of the IOBC (Sterk et al. 1999), spirotetramat can be categorized as harmless (cat. 1) to *C. montrouzieri* because laboratory trials showed no harm against this species. These results are based on both lethal (acute toxicity) and sublethal (fecundity, egg hatching and larval and pupal development) effects on adults and immature stages of *C. montrouzieri* when the insecticide was directly applied as well as when it was ingested through treated prey.

According to our data, spirotetramat is much less toxic than the two conventional insecticides used as treated controls in this study. Consequently, it could be used in IPM programs where coccinellids are key natural enemies as it is the case of Spanish citrus, where pyriproxyfen and chlorpyriphos are widely used against *A. aurantii* (Tena et al. 2011). However, as shown herein and in previous studies, these insecticides are toxic for *C. montrouzieri*, as well as for other natural enemies (Jacas and Urbaneja 2010).

Our results allow us to categorize the growth regulator insecticide, pyriproxyfen, as harmful (cat. 4) for larvae and adults of *C. montrouzieri*. Larvae either treated with this insecticide or fed with treated prey reached the pupal stage but most of them died during the metamorphosis and those that survived were sterile. Furthermore, even if adults fed on pyriproxyfen-treated prey survived and also had higher fecundity, their eggs never hatched as already reported by other authors (Hattingh and Tate 1995; Franco et al. 2004), as well as for other coccinellids (Mendel et al. 1994; Grafton-Cardwell and Gu 2003). Only

Cloyd and Dickinson (2006) observed that pyriproxyfen was harmless to the adult stage of *C. montrouzieri*. Nonetheless, their study was solely based on the mortality of *C. montrouzieri* adults 48 h after the spray. Therefore, our results confirm the importance of estimating sublethal effects in this kind of studies (Desneux et al. 2007). Galvan et al. (2005) also observed that spinosad did not affect the survival of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) adults, but reduced egg hatching.

In a previous study, Boyero et al. (2005) demonstrated that chlorpyriphos, contrary to pyriproxyfen, affected the survival of *C. montrouzieri* adults. In general, our study shows that chlorpyriphos is moderately toxic (cat. 3) for adults of *C. montrouzieri*. Although our results showed that chlorpyriphos did not affect adult survival, it reduced fecundity and egg hatching more severely than pyriproxyfen. Bellows and Morse (1988) found that the effect of the residues of chlorpyriphos on the survival of *C. montrouzieri* adults was lower than that of other insecticide (carbaryl, ethidathion, and parathion) even if they did not study the sublethal effects of these biocides.

Although there is little information about the side effects of spirotetramat on other natural enemies, the available studies have similarly categorized this lipid biosynthesis inhibitor as harmless to other natural enemies, such as the predators *Episyrphus balteatus* (Degeer) (Diptera: Syrphidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) (Schnorbach et al. 2008; Moens et al. 2011) and the parasitoids *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae), Coccidoxenoides perminutus Girault (Hymenoptera: Encyrtidae) and *Anagyrus* sp. Near pseudococci (Hymenoptera: Encyrtidae) (Mansour et al. 2011; Moens et al. 2012). Therefore, based on these results, and on the side effects observed in our study, spirotetramat could be included in IPM programs where *C. montrouzieri* is a key natural enemy. This is the case of crops such as citrus, grapes, and ornamentals (Daane et al. 2007; Mus¸tu et al. 2008; Urbaneja et al. 2015). In citrus, where *A. aurantii* is a key pest, spirotetramat is an alternative insecticide to chlorpyriphos and pyriproxyfen which are widely used. The use of spirotetramat against the first generation of *A. aurantii* at the end of spring would be compatible with augmentative releases of *C. montrouzieri* adults in the field. However, it would be wise to extend the studies on its side effects to other key beneficial insects in citrus, particularly on a relevant parasitoid such as *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) (Vacas et al. 2012), and a relevant predatory mite, such as *Euseius stipulatus* (Athias-Henriot) (Acari: Phytoseiidae) (Abad- Moyano et al. 2009).

Acknowledgments

We are grateful to Ramón Aparici and Alberto García (Insectario GVA) for maintenance of insect colonies. This research was partly funded by Conselleria d'Agricultura, Pesca I Alimentació from Generalitat Valenciana, the Spanish Ministry of Science and Innovation (Projects: AGL2008-05287-C04/AGR and AGL2011-30538-C03/AGR) and by Bayer CropScience by means of a cooperative agreement. L.P. was recipient of a grant from IVIA and A.T. of a postdoctoral fellowship from MCINN (Juan de la Cierva Program).

6.2 Lethal and sublethal effects of three pesticides used in citrus on the parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae)

Abstract

In the study, we assessed the lethal and sublethal effects of spirotetramat, spinosad and chlorpyrifos on the parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), the main parasitoid of *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) in citrus. These insecticides are used against *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae), a new major pest of citrus which management is based on chemical control. Chlorpyrifos and spinosad were classified as harmful for *A. melinus* adults because they died when they were in contact with fresh residues. Spirotetramat was moderately harmful against adults because, despite not affecting their longevity, it reduced their fertility. Chlorpyrifos was classified as moderately harmful for *A. melinus* immatures. On the other hand, spirotetramat resulted slightly harmful for immatures and spinosad was classified as harmless. Therefore, according to their side effects on *A. melinus,* spirotetramat can be recommended against *P. kellyanus* and spinosad could be an alternative to chlorpyrifos when immature of *A. melinus* predominate in the field.

Keywords. *Aonidiella aurantii*, citrus, IPM, *Pezothrips kellyanus, s*ublethal effects.

6.2.1 Introduction

The control of new invasive pests is primarily based on pesticides which are noxious to beneficial arthropods (Desneux et al. 2007) and might disrupt biological control of secondary pests. This is especially important in permanent ever-green crops, such as citrus, because pests and their natural enemies are active and abundant throughout the year. For this reason, accurate assessment of potential side effects of pesticides on natural enemies is critical for developing effective IPM strategies against the new pests (Desneux et al. 2006a; Stark et al. 2007). Many laboratory assays rely almost exclusively on the assessment of lethal effects. However, pesticides may induce multiple sublethal effects in individuals that survive an exposure to a given pesticide (Desneux et al. 2007), and these effects could have important impact on natural enemies population dynamics (Stark and Banks 2003). Sublethal effects could impair the physiology (e.g. neurophysiology, development, longevity, fecundity and sex-ratio) and the behavior (e.g. mobility, orientation, feeding, host searching, oviposition and mating) of natural enemies (Desneux et al. 2004a, b; Suma et al. 2009; Evans et al. 2010; Arnó and Gabarra, 2011; Saber, 2011; Stara et al. 2011; He et al. in press and see Desneux et al. (2007) for a thorough review). Studying more subtle endpoints (e.g. behaviors, Desneux et al. 2004c) and using multistep bioassays to evaluate the potential effects of pesticides on natural enemies is therefore required to assess risk in a more complete way (Desneux et al. 2006a, 2006c, 2007; Stark et al. 2007; Biondi et al. 2012, 2013).

Pezothrips kellyanus (Bagnall) (Thysanoptera: Thripidae) has become a new major pest in Spain citrus and other countries (Stevens et al. 2000; Webster et al. 2006; Baker 2006; Vassiliou 2007; Navarro et al. 2008;

Crisp and Baker 2009). This feeding habit of *P. kellyanus* nymphs causes patches or rings of scarred tissue around the fruit apex as it grows. This damage leads to economical losses due to reduced market value of the affected fruits and it is particularly severe on navel oranges, lemons (Conti et al. 2003; Varikou 2010) and grapefruits (Mound and Jackman 1998; Baker et al. 2002; Vassiliou 2007; Vassiliou 2010). As other invasive pests, its biological control is still under development (Baker 2011; Navarro-Campos et al. 2012b) and chemical control is, nowadays, the only alternative for growers. The insecticides chlorpyrifos and spinosad display a high efficacy against *P. kellyanus* nymphs and reduce the percentage of damaged fruits when a sole generation of *P. kellyanus* attacks the fruit (Planes et al., submitted). Spirotetramat does not display a knockdown effect and its efficacy against nymphs and to decrease damages is lower than chlorpyrifos and spinosad (Planes et al., submitted). However, a sole application of this insecticide at the end of May might control this thrips species and the California red scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), a key citrus pest in the Mediterranean basin (Tena et al. 2013a). Despite the importance of these insecticides on IPM of citrus, their side effects on key natural enemies of citrus pest are poorly known. Parasitoids are among the three main groups of natural enemies in citrus (Urbaneja and Jacas 2011). Herein, we assess the lethal and sublethal effects of spirotetramat, spinosad and chlorpyrifos on *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), the main parasitoid of *A. aurantii* and, therefore a representative parasitoid of citrus. In detail, we determine the effect of these insecticides on the mortality, longevity and fertility of *A. melinus* adults and on their immature mortality. These results will permit to establish an IPM program against *P. kellyanus* in citrus until a more environmental strategy will be developed.

6.2.2 Materials and methods

6.2.2.1 Pesticides and dosages

The insecticides used in these assays were chlorpyrifos, spinosad and spirotetramat (Table 6.2.1). The concentration of these different insecticides was the maximum authorized for citrus according to the label.

Table 6. 2. 1. Details of the insecticides and chlorpyrifos evaluated in the present study.

Active Ingredient	AI g/l	Trade name	Company	Concentration (ml/hl)
Chlorpyrifos 48 % [EC] w/v	480	Dursban-48	Dow Agrosciences Iberica, S.A.	200
Spinosad 48 % [EC] w/v	480	Spintor 480sc	Dow Agrosciences Iberica, S.A.	25
Spirotetramat 15 % [EC] w/v	150	Movento 150 OD	Bayer Cropscience, S.L.	50

6.2.2.2 Plants and insects

Aonidiella aurantii was obtained from a laboratory culture established at Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia, Spain, in 1999. This colony was initiated from scales collected in citrus fields in the vicinity of Alzira (Valencia, Spain) and refreshed every 2-3 years. *Aonidiella aurantii* was reared on green lemons. A 24-cm^2 area of the lemons was infested by exposing them to gravid female scales over 48 h. The remaining surface of the lemons was covered with wax to prevent fruit desiccation. Once infested, lemons were maintained at 27

± 1 °C, 70 ± 5% RH and darkness until female scales reached the third nymphal instar (19-22 days).

Less than 3-day-old adults of *A. melinus* were obtained from commercial mass rearing on pumpkins infested with *Aspidiotus nerii* Bouche (Hemiptera: Diaspididae) (*Aphytis melinus* 10,000; Koppert Biological Systems S.L Águilas, Murcia, Spain). Once in the lab, parasitoids were released in a rearing box (51 × 51 × 41 cm) until they were used the next day. The top of the box was impregnated with small lines of honey to feed parasitoids.

Clementine fully expanded leaves from the last flush (approximately 5 cm length) were collected from potted trees grown under controlled environmental conditions (25 ± 2 °C, 65 ± 10 % RH) in an insecticide-free greenhouse located at Instituto Valenciano de Investigaciones Agrarias Moncada (IVIA), Valencia, Spain. Artificial light was supplied to provide a 14:10 h L:D photoperiod.

6.2.2.3 Toxicity trials

Insecticides were applied to lemon fruits and clementine leaves using a Potter Spray Tower (Burkard Scientific Ltd, Uxbridge, UK). Two ml of the corresponding product dilution was sprayed at 150 kPa resulting in a deposit of 1.5 mg/cm^2. A distilled water spray control was included. Water was applied first in all the assays. Then, the pesticides were applied in a random order and, finally, chlorpyrifos. The experimental units containing the scales and parasitoids were kept in a climatic chamber until the end of the assay at 25 ± 1 °C, 60 ± 10 % RH and a photoperiod of 16:8 h (L:D) (the same climatic conditions were used in the following assays). The experimental unit consist of a 60 ml screw cap polystyrene container (38 x 65 mm) (Deltalab, SL, Barcelona, Spain) containing the treated leaf (Vanaclocha et al. 2013). The tube had a hole of 2 cm in diameter covered by gauze at the top for ventilation. The lid had a small hole to introduce the leaf petiole. The container was reversed and the lid rested on a floral foam piece drenched with water. To keep the leaf turgid, the petiole coming out of the lid hole was inserted in the soaked foam (Figure 6.2.1).

Figure 6. 2. 1. Experimental units used to evaluate the side effects of selected pesticides on *A. melinus* adults.

Adults of *A. melinus* were directly suctioned from the rearing box (Figure 6.2.2) described above into each experimental unit. To minimize the manipulation of the parasitoids, the experimental unit was assembled to the suction device designed specifically for this purpose (Vanaclocha et al. 2013).

Figure 6. 2. 2. Rearing box where parasitoids were released.

During the experiments, parasitoids were fed with a mixture of water-honey (3:1; v:v). This mixture was injected in a cylindrical cotton filter (1.5 cm x 0.7 cm diameter) attached to the external side of the ventilation hole. The filter was daily soaked with the water-honey mixture to avoid drying (Vanaclocha et al. 2012).

6.2.2.4 Side effects on parasitoid longevity

To determine the longevity of the survival females, two females from each previous replicate/container were individualized in a 1 cm diameter glass vial provisioned with a drop of honey on its inside wall and stoppered with a cotton plug. Glass vials were placed in climatic chambers. Survival was checked daily between 8:30 and 10:30 until they died and honey was renewed weekly. The mean survival of the two females was calculated for each replicate (experimental unit explained above). Ten replicates were carried per treatment.

6.2.2.5 Side effects on parasitoid fecundity

To determine the fecundity of the survival females, other two females and males of each replicate/container were introduced in a transparent 10 × 8 diam. glass container with one lemon infested with 80-120 third instars *A. aurantii*. The container was covered with muslin and enclosed with a plastic rubber. One 1.5 ml Eppendorf filled with water and sealed with cotton and a drop of honey on its inside wall were also provided. Glass containers were placed in climatic chambers. Five days later, alive parasitoids were removed and the containers with the lemons and scales were kept in the climatic chamber during 18 days to permit the emergence of the immatures. Twice a week, emerged parasitoids were removed and counted under a binocular. A total of seven replicates (lemons) were carried per each treatment.

6.2.2.6 Exposure to pesticides and lethal effect on immatures

To obtain immature *A. melinus,* lemons infested with 80-120 third instars *A. aurantii* were individually exposed to three 3 d-old females and two males in a glass container as the explained above during 48 hours. Four days later, the lemons were treated with the same products and concentrations using the Potter Spray Tower. As soon as lemons dried, they were individually introduced in another glass container and transferred to a climatic chamber during 18 days to permit adult emergence. Twice a week, emerged parasitoids were removed and counted under a binocular. A total of seven replicates (lemons) were carried per each treatment.

6.2.2.7 Data analysis

For each experiment, the mortality of *A. melinus* was compared by means of one-way variance analysis (ANOVA) at $P < 0.05$. When needed, data were angular transformed to achieve homoscedasticity and normal distribution of residues. The LSD test was applied for mean separation at $P < 0.05$. When significant differences were found between the control and the pesticide, mortality was corrected using the Abbott's formula (Abbott 1925).

To provide a single value summarizing potential deleterious effects of pesticide tested on adults, the toxic effects on adults and immatures (both lethal and sublethal effects) of each pesticide were also expressed as the Reduction Coefficient *E* (Urbaneja *et al*. 2008). For this, when significant differences were found between the control and the insecticide ($P < 0.05$), the corresponding reduction value (RV) was calculated using the Abbott formula (Abbott 1925). When no significant differences were found the RV was assigned as 1. Subsequently, the different RVs obtained for the same product in adult experiment (Urbaneja et al. 2008) were combined as follows:

$$E = 100 \times (1 - (\mathrm{RV}_{\text{Adult mortality}} \times \mathrm{RV}_{\text{Adult longevity}} \times \mathrm{RV}_{\text{Fertility}})$$

Each term represents the percentage reduction in relation to the control for the different parameters considered. The resulting value (E) was interpreted according to International Organization of Biological Control (IOBC) standards (Sterk et al. 1999), that include four categories (1) harmless; (2) slightly harmful, (3) moderately harmful and (4) harmful, which correspond to reductions below 30 %, between 31 and 79 %, between 80 and 99 % and higher than 99 %, respectively. The same analysis was carried out in the immature assay but based only on the number of adults emerged per treated lemon.

6.2.3 Results

6.2.3.1 Lethal effect on *Aphytis melinus* adults

Chlorpyrifos and spinosad caused a 100% of mortality on *A. melinus* adults and consequently were excluded from the following assays to measure sublethal effects and were classified as harmful for adults (IOBC class: 4; **Table 6.2.2**). On contrary, the mortality caused by spirotetramat did not differ from the control ($F_{1,18} = 3.83$; $P = 0.067$).

6.2.3.2 Side effects on *Aphytis melinus* longevity and fecundity

Spirotetramat did not affect the longevity of *A. melinus* females ($F_{1,\ 19}$ = 0.01; P = 0.91). However, their fecundity was negatively affected by spirotetramat after a 3-d exposure period ($F_{1,\ 16}$ = 9.46; P = 0.008) (Table 6.2.3). Therefore, spirotetramat was classified as moderately harmful for adults of *A. melinus* (IOBC class: 3).

6.2.3.3 Lethal effect on *Aphytis melinus* immatures

The number of adults that emerged from parasitized scales treated with chlorpyrifos and spirotetramat was significantly lower than from untreated scales ($F_{3,\ 27}$ = 9.41; $P < 0.001$) (Table 6.2.4). Among these two insecticides, the number of adults that emerged from of *A. melinus* treated with chlorpyrifos was significantly lower than those treated with spirotetramat. Therefore, spinosad was classified as harmless (IOBC class: 1), spirotetramat as slightly harmful (IOBC class: 2) and chlorpyrifos as moderately harmful (IOBC class: 3).

Table 6. 2. 2. Adult mortality (%), longevity (days), fecundity (pupae / two adult females searching during five days) and immature survival (adults emerged from hosts parasitized by three *A. melinus* females during two days and then treated five days later) of *Aphytis melinus* exposed to three insecticides and a water control.

	Chlorpyrifos	**Spinosad**	**Spirotetramat**	**Control**
Adult mortality	100.0 ± 0.0a [0]	100.0 ± 0.0a [1]	16.5 ± 2.0b [0.9]	10.6 ± 2.1c
Longevity	-	-	5.35 ± 1.2a [1]	5.5 ± 0.7a
Fertility	-	-	1.0 ± 0.5b [0.2]	5.0 ± 1.3a
E (%) (IOBC cat.)	100 (4)	100 (4)	82 (3)	-
Immature survival	0.2 ± 0.1c [0.1]	2.4 ± 0.6ab [0]	1.6 ± 0.5b [0.4]	3.8 ± 1.0a
E (%) (IOBC cat.)	90 (3)	0 (1)	60 (2)	

Reduction factors for each parameter appear in brackets, and the IOBC category corresponding to the total reduction value (E) is shown in the last row.

[a] Within a row, data followed by a different letter are significantly different (*P*<0.05, LSD test)

6.2.4 Discussion

Pezothrips kellyanus has become a new major pest in citrus and chemical control is, nowadays, the only alternative for growers. However, the use of insecticides against this pest might have severe side effects on the most prevalent natural enemies of citrus, where most of the pests are under natural control (Jacas et al. 2010). Here, we have evaluated the side effects of three insecticides that have been recently assessed against *P. kellyanus* in a companion manuscript on *A. melinus,* a representative parasitoid of citrus because it is the main natural enemy of *A. aurantii*. These insecticides were selected because they have different mode of action and they also can control *A. aurantii*.

Chlorpyrifos is one of the most-widely used active ingredients for pest control in citrus against hemipterans (scales and aphids) and thrips (Crisps et al. 2009; Morse and Grafton-Cardwell 2012a; Navarro- et al. 2012a; Planes et al. 2013). In our companion study, chlorpyrifos had a knockdown effect and reduced the percentage of fruits damaged by *P. kellyanus*. However, the use of this insecticide should not be recommended for two reasons. First, *P. kellyanus* might develop resistance to this insecticide as it has occurred in Australia if its chemical control relies on it (Baker *et al.*, 2004; Baker et al. 2009). Second, our results show that it is highly toxic for *A. melinus*. It resulted harmful for adults because they died when were in contact with fresh residues. Therefore, we could not determine the effect of chlorpyrifos on the longevity and fecundity of *A. melinus* females. This result is in agreement with previous studies, according to which chlorpyrifos is highly harmful to parasitoids (Davies and McLaren 1977; Morse and Bellows 1993; Hassan 1997; Jacas Miret and Garcia-Marí 2001; Michaud and Grant 2003; Boller et al. 2005; Suma et al. 2009;

Vanaclocha et al. 2013, González-Zamora et al. 2013) Similarly, it also resulted moderately harmful for immatures *A. melinus*. After spraying parasitized scales, no *A. melinus* adults emerged. These scales hold 4-6 days-old *A. melinus*. According to Abdelrahman (1974), *A. melinus* are larvae at the climatic conditions of this study. Adult stage of parasitoids is considered the most susceptible to insecticides (Sterk et al.1999) but immature can be also negatively affected (Desneux et al. 2007). These analyses are especially important when immatures are the predominant instar in the field as occur with *A. melinus* in spring (per. observations). To our knowledge, the effect of chlorpyrifos on immatures of *A. melinus* had been tackled only once in a preliminary study (Suma et al. 2009). These authors also encountered that chlorpyrifos resulted toxic, but the stage of the immatures in this study is unclear. This insecticide also results highly toxic against coccinelids (Planes et al. 2013) and, therefore, it should not be recommended under IPM in citrus.

The other tested insecticide, spinosad, has been identified as potential candidate for Integrated Pest Management (IPM) programs in citrus because of its fast action (insects to die of exhaustion within 1-2 days) and its low persistence (Thompson et al. 2000; Conti et al. 2001; Cisneros et al. 2002). Its residues on the leaf surface are broken down by sunlight in few days (Salgado et al. 1998). For these characteristics, it is recommended against *P. kellyanus* in Australia (Baker 2006) and against *Scirtothrips citri* (Moulton) for citrus in California (Immaraju and Morse 1991; Khan and Morse 2006; Morse and Grafton-Cardwell 2012b). In our study, spinosad resulted harmful for *A. melinus* adults after three days of exposure. As chlorpyrifos, the high mortality did not permit us to determine its effect on the longevity and fecundity of *A. melinus* females. Similarly, numerous studies have proved its toxicity to many Hymenopteran parasitoids in various formulations (Michaud

2003; Williams et al. 2003; Haseeb et al. 2004; Williams and Price 2004; Wang et al. 2005; Kumar et al. 2008; González-Zamora et al. 2013). These results should be analyzed with caution because under laboratory conditions and artificial light spinosad might not degrade as fast as it does in the field. Spinosad was classified as harmless for immatures and therefore it could be recommended when this is the main stage of *A. melinus* in the field.

Spirotetramat is active against a wide spectrum of sucking insects, including aphids, scales (soft and armored), mealybugs, whiteflies, psyllids and selected thrips species (Grafton-Cardwell et al. 2007) but its side effects are poorly known. According to our results, spirotetramat was moderately harmful against adults of *A. melinus* because, despite not affecting their longevity, it reduced its fertility. The lack of studies dealing with the side effects of spirotetramat does not allow us to compare our results. This insecticide interferes with lipid biosynthesis (IRAC 2012). Therefore, spirotetramat should be harmless for adult parasitoids because they do not synthesize lipids and they use the lipid reserves that contain at birth (Visser et al. 2010). Our results suggest they it might also affect egg maturation of sinovigenic parasitoids, which depends on the content of lipids (Casas et al. 2005). Interestingly, despite the high efficacy of spirotetramat against *A. aurantii* (Tena et al. 2013b), it resulted slightly harmful for its parasitoid *A. melinus* in this work. Therefore, the results presented herein, together with a recent study showing that it is harmless to the coccinelid *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinelidae) (Planes et al. 2013), indicate that spirotetramat could be recommend in citrus IPM programs. Especially, if a sole application of spirotetramat at the end of May controls *A. aurantii* and *P. kellyanus* when the population levels of the latter are low.

In conclusion, the results presented herein together with the companion manuscript (Planes et al., submitted) show that spinosad can be an alternative to chlorpyrifos against *P. kellyanus* when immature of *A. melinus* predominate in the field. This is because spinosad displays a knockdown effect against *P. kellyanus* and it is harmless for immatures of *A. melinus*. For this last reason, spirotetramat can be also recommended against *P. kellyanus* but, more interestingly and contrary to spinosad, this insecticide can be also used against *A. aurantii* with a sole application.

CAPÍTULO 7

Discusión General
y Conclusiones

Actualmente, la globalización ha facilitado el transporte de un gran número de plagas invasoras. Los cítricos españoles no son una excepción a esta tendencia y en las últimas décadas son numerosas las introducciones de nuevas plagas como ha ocurrido con el trips *Pezothrips kellyanus*. En la Cuenca Mediterránea se detectó su presencia en los años ochenta pero no se observaron daños hasta los noventa y en la Comunidad Valenciana hasta 2007 en la comarca de la Ribera Alta (Navarro et al. 2008b). Actualmente, *P. kellyanus* está distribuido por toda la Comunidad Valenciana y produce daños principalmente en la comarca de La Ribera-Alta y La Safor, siendo las zonas afectadas cada vez mayores.

Como en la mayoría de plagas invasoras, el único método de control efectivo que se ha desarrollado tras su entrada en nuestro país es el control químico, sin embargo todavía quedan muchas cuestiones por resolver para mejorarlo y alterar lo menos posible la fauna auxiliar existente en nuestros cítricos que actualmente están controlando otras plagas. Por todo ello se planteó esta tesis doctoral. En concreto, se decidió mejorar y facilitar el muestreo y la aplicación de productos fitosanitarios. Para ello se estudió la distribución de las ninfas de *P. kellyanus* en el árbol a lo largo del día durante la época que producen los daños. Además se determinó el número de generaciones que pueden atacar al fruto recién cuajado ocasionando daños en la piel y cuál de estas generaciones fue la más dañina. Una vez respondidas estas preguntas, se evaluó la eficacia de diversos insecticidas con diferente modo de acción para evitar la aparición de resistencias y si alguno de ellos es capaz de controlar, con un solo tratamiento, todos los ataques de ninfas de *P. kellyanus*. Por último, se evaluaron los efectos secundarios que estos tratamientos pueden tener sobre la fauna auxiliar de nuestros cítricos.

En general, los daños producidos por los trips en cítricos son de tipo estético. Las ninfas se alimentan de los frutos recién cuajados produciendo escarificaciones circulares alrededor del pedúnculo. Además, pueden producir otros tipos de daños como el plateado de los frutos maduros o la quemadura de las hojas entre otros (Lacasa et al. 1996; Navarro et al. 2008b). En la cuenca mediterránea, pueden encontrarse tres especies de trips que pueden causar daños importantes (Longo, 1986; Lacasa et al. 1996; EPPO, 2005). En el caso de nuestros cítricos, *P. kellyanus* es el principal trips (Navarro et al. 2008b; 2012a; 2012b). En los dos campos muestreados en esta tesis se han observado estos tipos de daños, si bien la presencia de frutos maduros plateados como consecuencia de la alimentación de las ninfas y adultos ha sido anecdótica. Por ello sólo se han valorado los daños producidos tras el cuajado del fruto y que dan lugar a las escarificaciones.

De los muestreos realizados semanalmente se determinó que **la dinámica de las poblaciones de *P. kellyanus* varía según parcelas, pudiendo tener una o dos generaciónes que dañan los frutos**. Esta es la primera vez que se ha determinado el número de generaciones o ataques que se pueden dar, no solo por *P. kellyanus*, sino tambien por otros trips en cítricos. En el trabajo de Navarro-Campos (2013) también se pueden observar dos máximos de ninfas, uno al principio de mayo y otro a finales, lo que coincidiría con los resultados de esta tesis. Estos resultados tienen gran importancia para el control de *P. kellyanus* en cítricos porque por una parte implica que los insecticidas utilizados tengan la persistencia suficiente para afectar a los dos ataques, y por otra parte, este resultado también implica que sea necesario realizar muestreos después del tratamiento contra la primera generación para determinar si es necesario un segundo tratamiento.

La presencia de una segunda generación de ninfas atacando los frutos en la parcela de Tavernes de la Valldgnia también influyó en el porcentaje de frutos dañados. Así, el porcentaje de frutos dañados en Tavernes de la Valldigna (~70%), donde se dieron dos generaciones, fue superior al observado en Alzira (~50%) a pesar de que en esta última parcela el porcentaje de frutos ocupados fue superior. Tras estudiar la influencia de la segunda generacion en los frutos en la parcela de Tavernes de la Valldigna, se determinó que los frutos ocupados por la primera generación presentaron principalmente daños ligeros mientras que los ocupados por la segunda generación presentaron un porcentaje similar de daños ligeros y severos. Esto sugiere que **la segunda generación de ninfas de *P. kellyanus*, a pesar de ser menos intensa, es más dañina que la primera.**

Una vez conocida la dinámica de las ninfas de P. kellyanus, se determinó su distribución en la copa del árbol a lo largo del día. Se observó que **las ninfas de *P. kellyanus* tienden a ocupar la mitad de la parte alta de la copa coincidiendo con la mayor presencia de frutos dañados en esa zona**. **No se observaron diferencias a lo largo del día ni entre orientaciones**, lo que simplifica el muestreo de la plaga, que se puede realizar a cualquier hora del día independientemente de la orientación. Actualmente se recomienda muestrear no menos de 300 frutos desde la caída de pétalos hasta que alcancen un diámetro de unos 4 cm, pues cuando alcanzan este tamaño rara vez pueden producirse daños de trips (Navarro-Campos et al. 2013). Sin embargo este muestreo puede suponer más de dos horas de trabajo, si se considera que para muestrear cada fruto se necesita unos 30 segundos. El muestreo de la parte alta, donde el número de ninfas es mayor, disminuiría el número de muestras y agilizaría el muestreo. Por la misma razón, los tratamientos deberían realizarse en la parte alta de la copa indiferentemente de la orientación, pese a que Vassilou (2010) observó que los adultos de *P. kellyanus* tendían a encontrarse en la zona norte y este de la copa del árbol.

En esta tesis se evaluó la eficacia de tres insecticidas que se seleccionaron por diversas razones. Clorpirifos se utilizó por su contrastada eficacia contra trips en cítricos. Spinosad, pese a no estar autorizado en esta formulación en cítricos, se incluyó por ser eficaz contra trips tanto en cítricos por resultados obtenidos en otros países como en otros cultivos y por ser respetuoso contra los enemigos naturales. La eficacia de spirotetramat contra trips es menos conocida pero puede ser un insecticida interesante por su alta eficacia contra otras plagas de cítricos, en especial el diaspídido *A. aurantii.* Una sola aplicación de spirotetramat contra trips podría ser eficaz también contra el diaspídido. Además su mayor persistencia podría controlar un segundo ataque.

Tras evaluar estos tres insecticidas se observó que **una sola aplicación de clorpirifos o spinosad fue suficiente para controlar la población de ninfas cuando sólo se dio un ataque de *P. kellyanus* sobre el fruto.** Ambos insecticidas han sido anteriormente evaluados contra esta plaga con resultados similares aunque variables (Benfatto et al. 2000; Purvis et al. 2002; Baker et al. 2004; Vassiliou 2007). Los trabajos anteriores, a diferencia de esta tesis, se basaron en la observación de los frutos dañados en campo pero en ningún caso se muestrearon las poblaciones de *P. kellyanus* ni antes ni después de los tratamientos. Por lo tanto, la falta de uniformidad en los resultados puede deberse a un posible segundo ataque

En nuestro ensayo en Tavernes de la Valldigna, donde se **observó la presencia de dos generaciones sobre los frutos, una sola aplicación de spinosad o clorpirifos no fue suficiente para evitar el segundo ataque de *P. kellyanus*. La persistencia de ambos productos aplicados contra la primera generación, tras la caída de pétalos, no pudo controlar la aparición de la segunda**. El porcentaje de frutos ocupados fue tres veces menor en esta segunda generación. Además, algunos frutos medían más de 4 cm durante este segundo ataque, aunque por el formato de esta tesis no se han presentado los resultados que mostraban el diámetro de los frutos. Por ello, es necesario continuar el muestreo tras el primer tratamiento y continuarlo hasta que el fruto tiene por lo menos 6,3 cm porque el porcentaje de frutos dañados fue superior al 50% en este ensayo donde no se trató contra la segunda generación. El umbral de tratamiento de esta generación, que es más dañina que la primera no se ha establecido, y nuestros resultados sugieren que el umbral establecido por Navarro et al. (2013) podría resultar insuficiente puesto que las poblaciones de ninfas nunca sobrepasaron este nivel. Cabe destacar, sin embargo, que Vassiliou et al. (2007) pulverizaron dos veces contra *P. kellyanus* y el porcentaje de frutos dañados fue de un 70%, por lo que una segunda aplicación no garantiza la reducción de los frutos dañados si no se realizan en los momento adecuados. Por lo tanto, esta segunda aplicación puede ser recomendada sólo cuando se muestrea la población de ninfas de *P. kellyanus*. Además, si es necesario un segundo tratamiento, los insecticidas empleados deberían tener un modo de acción diferente a los usados contra la primera generación para evitar resistencias y asegurar la eficacia de los mismos.

A pesar de que spinosad y clorpirifos han sido los productos más eficaces contra *P. kellyanus*, deben buscarse alternativas que eviten la aparición de resistencias en trips de cítricos, como ya se han encontrado en otros países (Morse y Brawner 1986, Immaraju et al. 1989). Por ejemplo *S. citri*, en California, que ha desarrollado resistencias a una larga lista de productos (Morse y Brawner 1986; Immaraju et al. 1989; Khan y Morse 1998). O en el sur de Australia, donde Baker et al. (2004) encontró que algunas poblaciones de *P. kellyanus* habían adquirido resistencia a clorpirifos.

En España, clorpirifos ha sido ampliamente usado para controlar *A. aurantii* y otras cochinillas durante las dos últimas décadas. Sin embargo, la elevada eficacia obtenida contra la primera generación de *P. kellyanus* en estos ensayos, sugiere que no ha desarrollado todavía resistencia a este insecticida. No obstante, para evitar la resistencia en poblaciones, los agricultores, deben evitar aplicar clorpirifos contra ambas generaciones de *P. kellyanus* o contra *A. aurantii* y *P. kellyanus* el mismo año. Para evitar estas resistencias, deben emplear los insecticidas solo cuando sea necesario, tras realizar los muestreos adecuados y implementar otros métodos de control incluidos en los programas de GIP (Urbaneja et al. 2015).

Además de los muestreos, el uso de insecticidas que respeten la fauna auxiliar existente en nuestros cítricos, es clave para mantener los programas de GIP actuales. **Spirotetramat** ha sido recientemente registrado contra *A. aurantii*, *Panonychus citri (McGregor) (Acari: Tetranychidae)* y thrips (Grafton-Cardwell et al. 2007; Grafton-Cardwell y Scott 2008; Morse y Grafton-Cardwell 2009; MAGRAMA 2014). A pesar que actualmente se está utilizando en California contra trips, **su eficacia fue menor que la observada para clorpirifos y spinosad en nuestro ensayos**. La falta de eficacia se debió principalmente a la falta de efecto de choque que si mostraron las otras dos materias activas. Por otra parte, a pesar de ser un insecticida sistémico, translaminar de larga persistencia (tres semanas aproximadamente) (Nauen et al. 2008), **no evitó el ataque de la segunda generación de *P. kellyanus*** en Tavernes de la Valldigna.

Durante los ensayos de campo se realizaron estudios para determinar el efecto de estos tratamientos sobre los principales grupos enemigos naturales presentes en los cítricos. En concreto, se intentó determinar el efectos sobre los tres principales grupos: fitoseidos, coccinélidos y parasitoides. Sin embargo, los resultados obtenidos en campo sólo nos permitieron estudiar los efectos de estos insecticidas sobre las poblaciones de fitoseidos. Aunque los fitoseidos no se llegaron a identificar a nivel de especies por el tipo de muestreo de observación directa en campo, se puede constatar que las poblaciones estaban compuestas principalmente por *Euseius stipulatus,* principal depredador del ácaro rojo *P. citri* (Ferragut et al. 1986).

Spinosad y spirotetramat disminuyeron el número de fitoseidos en campo. Kahn y Morse (2006) obtuvieron resultados similares en California con spinosad. Por lo tanto, si spinosad es finalmente registrado en cítricos para el control de trips se debería estudiar el efecto de estos tratamientos sobre las poblaciones del ácaro rojo que actualmente se encuentran controladas por *E. stipulatus*. En el caso de spirotetramat y pese a la toxicidad observada en campo, actualmente se está utilizando contra *A. aurantii* sin que hasta la fecha se haya detectado un incremento de las poblaciones de este ácaro fitófago en naranjos.

Para estudiar el efecto de estos tratamientos sobre los otros dos grupos de enemigos naturales se realizaron varios ensayos de laboratorio. De estos estudios se extrae que **clorpirifos no debería recomendarse, a**

pesar de su eficacia, por su alta toxicidad sobre los parasitoides y los coccinélidos. Estos resultados se ven reforzados por estudios coetáneos realizados también sobre el parasitoide *Aphytis melinus* (Vanaclocha et al. 2013; González-Zamora et al. 2013), si bien estos estudios no determinaron los efectos subletales y la mortalidad de los immaduros. De forma similar, **clorpirifos resultó tóxico sobre el coccinélido *C. montrouzieri*.** Hoy en día es bien conocido el efecto negativo que tienen los insecticidas que resultan tóxicos contra coccinélidos en los programas de GIP tanto por los científicos (Hattingh and Tate 1995; Mendel et al. 1994; Grafton-Cardwell y Gu 2003; Franco et al. 2004) como por los técnicos que han dejado de utilizar piriproxifén por su alta toxicidad sobre este grupo.

En el caso de spinosad, nuestros resultados sobre toxicidad deben analizarse con cautela porque este producto se degrada con la luz (Salgado et al. 1998) y en campo puede resultar menos tóxico de lo que sugieren nuestros resultados. De hecho, este insecticida está recomendado para el control de trips en California (Grafton-Cardwell et al. 2014) y Australia (Baker et al. 2014) por su alta eficacia y baja toxicidad sobre los enemigos naturales.

Por todo lo expuesto anteriormente, para mejorar la gestión de *P. kellyanus* sería recomendable realizar un muestreo semanal desde la caída de pétalos en la parte alta de la copa del árbol, y tratar en el momento que se supere el 6,25% de frutos ocupados por ninfas. Dos semanas después repetir el muestreo, para comprobar la evolución de la plaga por si se diese un segundo ataque. En caso de darse un segundo ataque de ninfas de *P. kellyanus* sería recomendable aplicar un insecticida con diferente modo de acción al aplicado anteriormente. En caso que este segundo ataque coincida con los tratamientos contra *A.*

aurantii, se podría recomendar aplicar spirotetramat, que pese a no ser tan efectivo contra *P. kellyanus,* nos permitiría reducir el número de tratamientos. Por último, es necesario buscar otros métodos de control más respetuosos así como más materias activas específicas contra trips que sean eficaces y respecten a los enemigos naturales como ha ocurrido recientemente con spirotetramat contra *A. aurantii* o pimetrozina y flonicamida contra los pulgones.

CAPÍTULO 8

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