UNIVERSIDAD POLITÉCNICA DE VALENCIA

ESCUELA TÉCNICA SUPERIOR DE INGENIEROS AGRÓNOMOS

DEPARTAMENTO DE PRODUCCIÓN VEGETAL

Gestión integrada de la araña roja *Tetranychus urticae* Koch (Acari: Tetranychidae): optimización de su control biológico en clementinos

TESIS DOCTORAL

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Memoria presentada por Poliane Sá Argolo

Para optar al grado de Doctora Ingeniera Agrónoma

Vº Bº de los directores

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"Se você quer ser bem sucedido, precisa ter dedicação total, buscar seu último limite e dar o melhor de si mesmo."

Ayrton Senna

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ABSTRACT

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most injurious pests affecting clementine orchards in Spain. In clementine trees, *T. urticae* inhabits the lower side of leaves where it produces silky webbing and dense colonies. It sucks the cell contents, causing chlorotic spots on the upper side of the leaves. At the end of summer, *T. urticae* infestations can result in characteristic fruit scarring and consequently fruit downgrading. To date, the control of *T. urticae* has been mainly based on chemical control. However, the use of this method is not always effective. Beneficial arthropods can be decimated, creating conditions favorable for uncontrolled proliferations of *T. urticae* and other pests. Currently, the integrated pest management programs in citrus aims to maximize the use of biological control. Thus, the overall objective of this thesis has been the improvement of the biological control of *T. urticae* in clementine orchards.

Biological control is not common in citrus nurseries where chemical control is prevalent. The systemic neonicotinoid imidacloprid applied as a drench is effective against three out of four key pests of young clementine plants in Spain - aphids, leafminers and scales. However, mites, the fourth key pest, are not controlled by imidacloprid and could be regulated by introduction of the predatory phytoseiid mites *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor). The aim of this study was to evaluate the effects of imidacloprid applied as a drench on the demographic parameters of these two predatory mites and the compatibility of *P. persimilis* releases with imidacloprid to control key pest populations in young clementine plants under field conditions. The results showed that some demographic parameters of *P. persimilis* were affected by imidacloprid. However, their combined effect on its intrinsic rate of increase was neutral. In contrast, imidacloprid negatively affected the demographic parameters of *N. californicus*. Field results proved that young clementine plants could be satisfactorily protected against key pests with releases of *P. persimilis* combined with drench applications of imidacloprid. The combination of imidacloprid with *P. persimilis* releases was highly effective for management of the key pests of young clementine plants in the nursery.

Conservation and augmentative biological control strategies have been developed to take full advantage of the natural enemies that occur in Spanish citrus orchards. Among them,

the predatory mites *E. stipulatus* (Athias-Henriot), *N. californicus* and *P. persimilis* play an important role in the biological control of tetranychid mites. However, these predatory mites are often affected by pesticides and information about the side-effects of these products against these beneficial arthropods is essential to guarantee the efficacy of these beneficial arthropods. The side-effects of some pesticides remain unknown and the primary aim of this study is to fill this gap. We have further used this information and that collected from other sources to compare the response of these three mite species to pesticides. Based on this information, *E. stipulatus* has resulted as the most tolerant species, followed by *N. californicus* and *P. persimilis*. Therefore, using *E. stipulatus* as an indicator species in citrus may have led to the paradox of selecting presumed selective pesticides resulting in excessive impact on *N. californicus* and, especially on *P. persimilis*. Because these two latter species are considered key for the biological control of *T. urticae* in citrus in Spain, we propose to use *P. persimilis* as the right indicator of such effects in citrus instead of *E. stipulatus*. This change could have a dramatic impact on the satisfactory control of Tetranychid mites in citrus in the near future.

Cover crops can serve as a reservoir of natural enemies by supplying alternative food sources as pollen. In turn, pollen quality and availability can modulate phytoseiid communities. In clementine trees associated with a cover crop of *Festuca arundinacea* Schreber, these communities were more diverse than those associated with a multifloral wild cover crop. As a consequence, the former had a better regulation of *T. urticae* populations than the latter. Longer provision of higher quality pollen in the multifloral cover relative to *F. arundinacea* is suspected to interfere with the biological control of *T. urticae* by specific phytoseiid predators (*P. persimilis* and *N. californicus*) by enhancing the less efficient generalist pollen feeder *Euseius stipulatus* which is a superior intraguild predator. To determine whether pollen quality is behind these results, the effect of the provision of two different pollens (*Carpobrotus edulis* (L.) L. Bolus and *F. arundinacea*) on the efficacy of two phytoseiid species (*E. stipulatus* and *N. californicus*) to regulate *T. urticae* populations has been studied under semi-field conditions. Results suggest that pollen provision does not enhance the ability of these phytoseiids to reduce *T. urticae* populations. However, *C. edulis* pollen resulted in explosive increases of *E. stipulatus* numbers that did not occur with *F. arundinacea* pollen. Therefore, poor quality pollen may prevent pollen feeders from reaching high numbers in the field. This effect could benefit phytoseiid species suffering intraguild predation by *E. stipulatus* explain field results.

Biological control through augmentative releases is a common practice against some citrus pests such as *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), *Planoccocus citri* Risso (Hemiptera: Pseudococcidae), and is developing in other species such as *Tetranychus urticae*. In the case of the *T. urticae*, releases of the phytoseiid mites *P. persimilis* and *N. californicus* resulted successful in nurseries. However, under field conditions, phytoseiids are not always detected after releases and control of *T. urticae* has not always been satisfactory, especially in the case of *N. californicus*. There is no information on the behavior of these phytoseiid mites in citrus orchards. Understanding their dispersal and preying activity could be useful to improve augmentative releases. Results showed that the phytoseiids exhibited a similar spatial distribution pattern under different prey densities. Both phytoseiids were predominantly found on leaves, where *T. urticae* colonies were present. However, at low pest densities, *N. californicus* showed a higher tendency to move to the trunk. Furthermore, prey detection by molecular methods proved *P. persimilis* to be a superior predator, even at lower prey densities. Based on these results, hot spot augmentative releases of *P. persimilis* are recommended.

RESUMEN

La araña roja, *Tetranychus urticae* Koch (Acari: Tetranychidae) es una de las plagas más perjudiciales que afectan los huertos de clementinos en España. En los clementinos, *T. urticae* habita en el envés de las hojas, donde produce tela y colonias densas. Absorbe el contenido de las células, causando manchas cloróticas en el lado superior de las hojas. Al final del verano *T. urticae* puede provocar en los frutos cicatrices características y en consecuencia, pérdida de su valor comercial. Hasta la fecha, el control de *T. urticae* se ha basado, principalmente, en el control químico. Sin embargo, este método no es siempre eficaz. Los artrópodos beneficiosos se eliminan, y se crean condiciones favorables para la proliferación incontrolada de *T. urticae* y otras plagas. En la actualidad, los programas de gestión integrada de plagas en cítricos, tienen como objetivo maximizar el uso del control biológico. En este sentido, el objetivo general de esta tesis ha sido la mejora del control biológico de *T. urticae* en los huertos de clementinos.

El control biológico no es común en los viveros de cítricos donde el control químico es frecuente. El neonicotinoide sistémico imidacloprid aplicado vía riego es eficaz contra tres de las cuatro principales plagas de plantones de clementino en España - áfidos, minador y cochinillas. Sin embargo, los ácaros, la cuarta la plaga clave, no se controlan con imidacloprid, pero podrían regularse mediante sueltas de los ácaros depredadores fitoseidos *Phytoseiulus persimilis* Athias-Henriot y *Neoseiulus californicus* (McGregor). El objetivo de este estudio fue evaluar los efectos de imidacloprid aplicado vía riego en los parámetros demográficos de estos dos ácaros depredadores y su compatibilidad con sueltas de *P. persimilis* para el control de las poblaciones de las plagas clave en plantones de clementino en condiciones de campo. Los resultados mostraron que algunos de los parámetros demográficos de *P. persimilis* se vieron afectados por imidacloprid. Sin embargo, su efecto combinado sobre la tasa intrínseca de incremento fue neutro. Por el contrario, imidacloprid afectó negativamente los parámetros demográficos de *N. californicus*. Los resultados de campo demostraron que los plantones de clementino podrían ser protegidos satisfactoriamente contra las plagas clave mediante liberaciones de *P. persimilis* combinadas con aplicaciones de imidacloprid vía riego. La combinación de imidacloprid con sueltas de *P. persimilis* fue muy eficaz en la gestión de las principales plagas de clementinos en vivero.

Las estrategias de control biológico por conservación y aumentativo se han desarrollado para sacar el máximo provecho de los enemigos naturales que aparecen en los huertos de cítricos españoles. Entre ellos, los ácaros depredadores *Euseius stipulatus* (Athias-Henriot), *N. californicus* y *P. persimilis* juegan un papel importante en el control biológico de los ácaros tetraníquidos. Sin embargo, estos ácaros depredadores son a menudo afectados por los plaguicidas y la información sobre los efectos secundarios de estos productos contra estos artrópodos beneficiosos es esencial para garantizar la eficacia de estos artrópodos. Los efectos secundarios de algunos plaguicidas siguen siendo desconocidos y el objetivo principal de este estudio es completar este hueco. Además, hemos utilizado esta información y la obtenida en otras fuentes para comparar la respuesta de estas tres especies de ácaros a los plaguicidas. Basándonos en esta información, *E. stipulatus* se ha confirmado como la especie más tolerante, seguida por *N. californicus* y *P. persimilis*. Por lo tanto, utilizar *E. stipulatus* como especie indicadora en los cítricos puede haber dado lugar a la paradoja de seleccionar plaguicidas presuntamente selectivos con un gran impacto sobre *N. californicus* y, especialmente *P. persimilis*. Debido a que estas dos últimas especies se consideran clave para el control biológico de *T. urticae* en cítricos en España, se propone el uso de *P. persimilis* como el correcto indicador de estos efectos en cítricos en lugar de *E. stipulatus*. Este cambio podría tener un gran impacto en el adecuado control de los ácaros tetraníquidos de los cítricos en un futuro próximo.

Las cubiertas vegetales pueden servir como reservorio de enemigos naturales mediante el suministro de fuentes de alimento alternativo como es el polen. A su vez, la calidad y la disponibilidad del polen pueden modular las comunidades fitoseidos. En los árboles de clementino asociados con la cubierta vegetal *Festuca arundinacea* Schreber, estas comunidades son más diversas que las asociadas a una cubierta espontánea multifloral. Como consecuencia, la primera proporcionó una mejor regulación de las poblaciones de *T. urticae* que la segunda. La disponibilidad de un polen de elevada calidad en una cubierta multifloral respecto al de *F. arundinacea* se sospecha que puede interferir negativamente con el control biológico de *T. urticae* mediante depredadores fitoseidos específicos (*P. persimilis* and *N. californicus*) aumentando las poblaciones del palinófago generalista *E. stipulatus*, que es un depredador intragremial superior. Para determinar si la calidad del polen está detrás de estos resultados, el efecto del suministro de dos tipos de pólenes

(*Carpobrotus edulis* (L.) L. Bolus y *F. arundinacea*) en la eficacia de dos especies de fitoseidos (*E. stipulatus* y *N. californicus*) para regular las poblaciones de *T. urticae* ha sido estudiada bajo condiciones de semi-campo. Los resultados sugieren que el suministro de polen no mejora la capacidad de estos fitoseidos para reducir las poblaciones de *T. urticae*. Sin embargo, el polen de *C. edulis* dio lugar a aumentos explosivos de *E. stipulatus*, lo que no ocurrió con el polen de *F. arundinacea*. Por lo tanto, el polen de mala calidad puede prevenir que especies palinófagas alcancen densidades excesivas en condiciones de campo. Este efecto podría beneficiar a las especies de fitoseidos que sufren la depredación intragremial por *E. stipulatus*, lo que explicaría los resultados de campo.

El control biológico mediante sueltas aumentativas se está convitiendo en una práctica cada vez más habitual en el cultivo de los cítricos, por ejemplo, contra *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), *Planoccocus citri* Risso (Hemiptera: Pseudococcidae). En otros casos, como *Tetranychus urticae*, está en desarrollo. Para esta especie, las sueltas de los fitoseidos *P. persimilis* y *N. californicus* resultaron muy eficaces en condiciones de vivero. Sin embargo, en condiciones de campo, no siempre es posible recuperar a los fitoseidos tras las sueltas y el control de *T. urticae* no siempre ha sido satisfactorio, especialmente en el caso de *N. californicus*. Hasta la fecha, no existe información sobre el comportamiento de estos fitoseidos en huertos de cítricos. De cara a mejorar las sueltas aumentativas de estos fitoseidos, sería importante entender sus pautas de dispersión y de actividad depredadora. Nuestros resultados han mostrado que ambos fitoseidos presentan pautas de distribución similares para distintas densidades de presa. Ambos fitoseidos se encontraron predominantemente en hojas, donde se encuentran las colonias de *T. urticae*. Sin embargo, a baja densidad de presa, *N. californicus* mostró una mayor tendencia a abandonar la hoja para dirigirse al tronco. Además, la detección de presa mediante técnicas moleculares nos indicó que *P. persimilis* es un depredador más eficiente, incluso a bajas densidades de presa. Basándonos en estos resultados, en clementinos, sería recomendable hacer sueltas de *P. persimilis* en focos.

RESUM

L'aranya roja, *Tetranychus urticae* Koch (Acari: Tetranychidae) està considerada el tetraníquid més perjudicial dels horts de clemetins a Espanya. *Tetranychus urticae* presenta una gran tendència a l'agregació i un alt potencial reproductiu. Viu a la cara inferior de les fulles on forma colònies protegides dels depredadors, acaricides i condicions climàtiques adverses, per la gran quantitat de teranyina que produeix, la qual cosa en dificulta el control i li permet estar particularment adaptada a ambients càlids i secs. La seua activitat alimentícia altera la respiració i la transpiració normals de la planta. Les fulles infestades, a la zona afectada, es tornen d'un color rovellat, i formen un concavitat característica que coincideix amb un abombament de la cara superior de la fulla, que també pren un color groguenc. En èpoques de l'any que l'arbre està sotmès a estrès hídric, si es produeixen elevades infestacions d'aquest fitòfag, es poden produir fortes defoliacions. A més, a finals d'estiu, *T. urticae* pot provocar cicatrius característiques que s'inicien a la zona estilar o peduncular i que en deprecien el valor comercial. Actualment, en els programes de gestió integrada de plagues en cítrics, la recerca s'ha dirigit a la implementació de mesures alternatives de control de *T. urticae*, com el control biològic. L'objectiu global d'aquesta tesi ha estat la millora del control biològic de *T. urticae* en clementí com una d'aquestes mesures racionals.

Dins de la gestió integrada de plagues, el coneixement dels efectes secundaris dels pesticides sobre la fauna útil és essencial per a poder compatibilitzar les pràctiques actuals d'ús de pesticides amb la conservació i les amollades augmentatives dels enemics naturals. Se sap que imidacloprid, un insecticida neonicotinoide, és molt eficaç contra pugons i la minadora de les fulles dels cítrics quan s'aplica pel reg, però no ho és contra *T. urticae*, que es controla principalment amb acaricides. Per aquesta raó, com a primer punt s'han realitzat estudis de laboratori i en vivers sobre la compatibilitat de l'ús del neonicotinode imidacloprid en aplicacions pel reg sobre els paràmetres demogràfics i les amollades inoculatives dels fitosèids *Neoseiulus californicus* (McGregor) i *Phytoseiulus persimilis* Athias-Henriot. En condicions de laboratori, alguns paràmetres avaluats en *P. persimilis* van resultar afectats positivament per imidacloprid. Contràriament, imidacloprid va afectar negativament els paràmetres de *N. californicus.* Els resultats de camp van demostrar que la combinació

d'imidacloprid amb amollades de *P. persimilis* va ser molt eficaç en la gestió de les principals espècies plaga de plançons de clementí en condicions de viver.

A continuació, es van estudiar els efectes secundaris d'alguns pesticides recomanats en cítrics sobre els depredadors *E. stipulatus*, *N. californicus* i *P. persimilis*, que fins ara es deconeixien. Els resultats van mostrar que l'oli mineral, etoxazol i spirotetramat, van ser selectius per a aquests tres depredadors. No obstant, l'abamectina va ser moderadament perjudicial i lleugerament persistent, mentre que etofenprox, va resultar ser el pesticida més perjudicial i persistent per a aquests fitosèids. A més, després de completar la llista dels efectes secundaris dels pesticides s'ha vist que *E. stipulatus* és l'espècie més tolerant, seguit de *N. californicus* i *P. persimilis.* Com que aquestes dues últimes espècies es consideren clau per al control biològic de *T. urticae* en els cítrics a Espanya, es proposa l'ús de *P. persimilis* com a indicador d'aquests efectes en cítrics, en comptes de *E. stipulatus*.

Els estudis realitzats sobre el paper del pol·len en el control biològic de *T. urticae* han demostrat que, en condicions de semi-camp, l'addició de pol·len de *Carpobrotus edulis* (L.) L. Bolus i de *Festuca arundinacea* Schreber, independentment de la seua qualitat, no augmenta la capacitat d'*E. stipulatus* ni de *N. californicus* per a reduir les poblacions d'aranya. Malgrat tot, la presència d'un pol·len de menor qualitat, en aquest cas el de *F. arundinacea*, pot evitar que els fitosèids que en depenen no incrementen el seu nombre, com és el cas d'*E. stipulatus*. Com a conseqüència, la pressió depredadora intragremial que aquest exerceix sobre les altres espècies de fitosèids més eficients en el control de l'aranya disminuiria. Aquest resultat és una possible explicació del control més satisfactori de *T. urticae* que s'observa en mandariners clementins associats a una coberta de la gramínia *F. arundinacea*.

Per a conèixer la distribució espacial dels fitosèids *P. persimilis* i *N. californicus* en plançons de clementí, es var fer amollades en plantes joves de clementí. Els resultats van indicar que aquests depredadors presenten diferents pautes de distribució espacial, quan s'exposen a diferents densitats de *T. urticae*. *P. persimilis* ha estat trobat predominantment a les fulles, independentment de la densitats de presa, mentre que en el cas de *N. californicus*, malgrat haver estat més abundant en fulla, en condicions de baixa disponibilitat de presa va mostrar una tendència a fugir cap al troc. La detecció de presa mitjançant tècniques moleculars va indicar que *P. persimilis* és un depredador més eficient, fins i tot a baixes densitats de presa.

A partir d'aquests resultats, en clementí, seria recomanable fer amollades de *P. persimilis* per focus.

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INTRODUCCIÓN

1.1. Citricultura

1.1.1. Importancia económica

En la actualidad, España ocupa el sexto lugar mundial de productores de cítricos (junto con Brasil, Estados Unidos, India, China y México), el segundo en producción de mandarinas y el mayor exportador de limón del mundo (FAOSTAT 2010).

Con aproximadamente 320.000 hectáreas cultivadas de cítricos, España produjo en la campaña de 2010, 6.092.435 millones de toneladas de cítricos. Alrededor del 45% de esta producción se destinó a los mercados de exportación, un 31% al consumo en fresco y un 24% al procesado (zumos y otros subproductos). La producción de cítricos españoles se distribuye en naranjas dulces (47%), mandarinas (38%) y limones (13%) (MAGRAMA 2010) (Fig. 1.1). Entre ellos, la naranja dulce es el principal producto destinado al consumo en fresco.

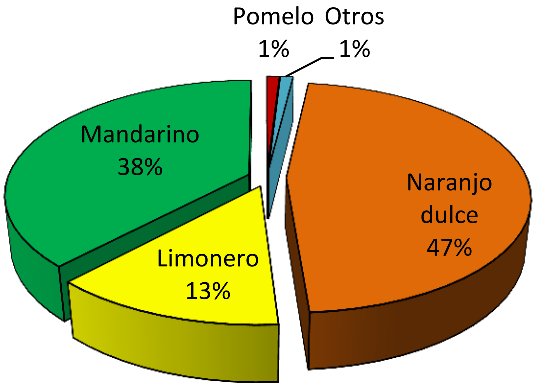

Fig. 1.1. Distribución de la producción de cítricos en España en 2010 (MAGRAMA 2010).

La mayor parte del área de producción citrícola se concentra en tres Comunidades Autónomas: Andalucía, Comunidad Valenciana y Murcia (MAGRAMA 2010). La Comunidad Valenciana, con 177.157 hectáreas dedicadas a este cultivo, es responsable del 64% de la producción y destaca como el mayor productor y exportador de mandarina y naranja dulce del país (MAGRAMA 2010). En 2011, la exportación de cítricos de la Comunidad Valenciana alcanzó los 1.903 millones de euros. Estos datos reflejan la importancia del sector citrícola en la agricultura valenciana y en su comercio exterior (IVEX 2012).

1.1.2. Gestión integrada de plagas en cítricos

La mayoría de las plagas descritas en los cítricos españoles están reguladas por la acción de sus enemigos naturales (depredadores, parasitoides y entomopatógenos), tanto autóctonos como naturalizados, que consiguen un excelente control biológico. Sin embargo, el control biológico de algunas plagas, como es el caso de la araña roja *Tetranychus urticae* Koch (Acari: Tetranychidae), el piojo rojo de California *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) y la mosca Mediterránea de la fruta *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), es aún insuficiente (Jacas & Urbaneja 2008). Estas especies son conocidas principalmente por causar daños en los frutos, en la mayoría de los casos cosméticos. Por ello, ya que la producción de cítricos españoles se destina mayoritariamente al consumo en fresco (84,9%) están sujetas a umbrales económicos de daños (UED) muy bajos (Hare 1994). Esto, sumado a un control biológico insuficiente, hace que en la mayoría de los casos sus poblacionales rebasen el UED atribuyéndoles el estatus de plagas clave (Jacas et al. 2010). Por lo tanto, es necesario tomar medidas adicionales para que esto no ocurra y que el producto final responda a la alta calidad exigida por el mercado (Jacas et al. 2010; Jacas & Urbaneja 2010).

Para que estas plagas no rebasen el UED, actualmente su control está basado mayoritariamente en el control químico (Urbaneja et al. 2008). Sin embargo, ésta es una práctica de control que ya no ofrece una solución satisfactoria, ya que su uso generalizado conlleva serios problemas a la agricultura en general, y a la citricultura, en particular, incluyendo: (i) la aparición de resistencias, (ii) la proliferación incontrolada de otras plagas como consecuencia de la reducción y/o eliminación de sus enemigos naturales y (iii) la presencia de residuos en el fruto, depreciando su valor comercial. Todo ello, desemboca en una estrategia de control costosa tanto para los agricultores como para el medio ambiente (Urbaneja et al. 2008).

Por estas razones y en virtud de lo dispuesto en la Directiva Europea 2009/128/CE del Parlamento Europeo y del Consejo de 21 de octubre de 2009 (EU 2009), las investigaciones se dirigen a un uso sostenible de los plaguicidas mediante la reducción de los riesgos y los efectos en la salud humana y el medioambiente. En este escenario, la Gestión Integrada de Plagas (GIP) y el desarrollo de estrategias o tácticas alternativas a las químicas pueden ser cruciales (EU 2009).

El hecho de que la araña roja *T. urticae* no sea considerada plaga clave en cítricos en la mayoría de los países productores de todo el mundo conlleva la existencia de un escaso número de estudios dirigidos a la gestión de esta especie en cítricos. La aplicación correcta de un programa GIP en cítricos 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. Es sobre estos puntos donde se centran los objetivos de esta tesis doctoral.

1.2. La araña roja, *Tetranychus urticae*

Tetranychus urticae Koch (Fig. 1.2) es una plaga cosmopolita y muy polífaga que ataca a numerosos cultivos de importancia económica, como los cultivos hortícolas, extensivos (algodón, maíz, etc.), cítricos, vid, frutales y ornamentales (Moraes & Flechtmann 2008). Este fitófago, conocido vulgarmente como araña roja, es uno de los ácaros tetraníquidos más perjudiciales que afectan a los huertos de cítricos en España, principalmente clementinas y limoneros (Jacas & Urbaneja 2008; Abad-Moyano et al. 2008; Jacas & Urbaneja 2010; Abad-Moyano et al. 2010a; Aguilar-Fenollosa et al. 2011a; Aguilar-Fenollosa et al. 2011b; Aguilar-Fenollosa et al. 2011c).

1.2.1. Clasificación Taxonómica

La clasificación taxonómica de *Tetranychus urticae* es la siguiente:

Reino: Animalia
Filo: Arthropoda
Clase: Arachnida
Subclase: Acari
Orden: Prostigmata
Familia: Tetranychidae
Género: *Tetranychus*
Especie: *T. urticae* Koch (1836)

1.2.2. Biología y Ecología

Tetranychus urticae es un ácaro fitófago con alto potencial reproductivo, ciclo de vida corto, tasa de desarrollo rápido y capacidad para dispersarse rápidamente. Su tamaño oscila entre 0,4 y 0,6 mm, en el caso de la hembra adulta, que tiene un aspecto globoso. El macho es más pequeño y aperado. Este ácaro puede presentar diferentes características morfológicas, sobre todo su color puede variar en respuesta a su régimen alimenticio, factores ambientales, planta huésped y estado de desarrollo. Esto ha provocado que le asignen diversos nombres a esta especie, entre los cuales están: *Tetranychus telarius* (L.), *T. bimaculatus* Harvey y *T. cinnabarinus* Boisduval. Incluso, algunos taxónomos consideran todavía que *T. urticae* y *T. cinnabarinus* son la misma especie (Dupont 1979; Meyer 1987), mientras que otros creen que son dos especies distintas (Zhang & Jacobson 2000; Zhang 2003), aunque en los foros científicos se prefiere considerarla una única especie.

Tetranychus urticae se reproduce mediante partenogénesis de tipo arrenotoca en la que los machos se desarrollan a partir de huevos no fertilizados (haploides), mientras que las hembras se desarrollan a partir de huevos fecundados (diploides). Esta especie presenta una proporción de sexos entre 2:1 y 9:1 a favor de las hembras (Macke et al. 2011). Cada hembra adulta puede poner unos 100-120 huevos, con una tasa de 3-5 huevos por día. Sin embargo, estas cifras pueden variar según la cantidad y la calidad del alimento, o las condiciones ambientales (Zhang 2003). Tiene un ciclo de vida corto que consta de cinco fases de desarrollo (huevo, larva, protoninfa, deutoninfa y adulto). Entre cada fase hay una fase inactiva o período quiescente, en la que adoptan una posición característica, recibiendo el nombre de *crisalis* (protocrisalis, deutocrisalis y deutocrisalis) (Fig. 1.3). La quiescencia está delimitada por el desprendimiento de las exuvias (Moraes & Flechtmann 2008; Badii et al. 2011). *Tetranychus urticae* en condiciones óptimas (~ 30°C) completa su ciclo en 9 días (Herbert 1981; Carey & Bradley 1982).

Fig. 1.2. Huevos y hembras adultas de *T. urticae*.

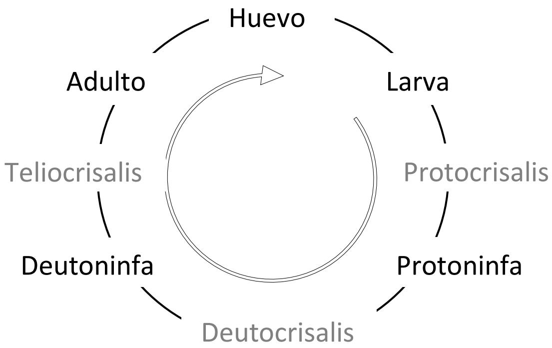

Fig. 1.3. Ciclo de vida de *T. urticae*.

Este ácaro tiene alta tendencia agregativa y desarrolla sus colonias en el envés de las hojas donde producen tela en abundancia que les protegen de los depredadores, acaricidas y condiciones climáticas adversas. Además, la tela también se utiliza como mecanismo de dispersión. En condiciones de escasez de alimento o cuando la planta está fuertemente infestada, los individuos se acumulan en el extremo de la hoja o del brote y después por corriente de aire o por gravedad son transportados a otra planta. *Tetranychus urticae* también puede vivir sobre los frutos cuando éstos están presentes (Moraes & Flechtmann 2008; Badii et al. 2011).

Temperaturas elevadas y condiciones de baja humedad favorecen el incremento de sus poblaciones que pueden alcanzar niveles perjudiciales y causar graves daños a las plantas hospederas. En climas fríos, este ácaro presenta baja actividad, mientras que en los países mediterráneos, donde la temperatura es suave, esta araña puede estar activa durante todo el año (García-Marí et al. 1991; García-Marí & Ferragut 2002; Aucejo-Romero 2005).

1.2.3. Daños

Tetranychus urticae es una de las especies más dañinas que atacan a los huertos de cítricos en España, sobre todo a los mandarinos clementinos (*Citrus clementina* Hort. ex Tan.), que es una variedad particularmente sensible al ataque de este ácaro (Aucejo-Romero et al. 2003; Bruessow et al. 2010; Abad-Moyano et al. 2010a).

El daño causado por este fitófago se debe a su actividad alimenticia. Para alimentarse *T. urticae* inserta sus estiletes en el tejido de la hoja, succionando el contenido de las células epidérmicas y parenquimáticas. El vaciado causa el colapso y muerte de las células que originan manchas cloróticas en las hojas (Fig. 1.4), disminuyendo la tasa de transpiración y la actividad fotosintética de la planta (Garrido & Ventura 1993; Park & Lee 2002; Aucejo-Romero et al. 2004; Martínez-Ferrer et al. 2006). Si la infestación coincide con altas temperaturas y/o estrés hídrico, puede causar defoliaciones graves y el número de flores producidas puede reducirse considerablemente. Cuando el ataque se produce sobre los frutos, ocasionan manchas herrumbrosas y difusas, que se inician en la zona estilar o peduncular (Fig. 1.5). Estas manchas causan un daño cosmético que reduce su valor comercial, produciéndose en consecuencia importantes pérdidas económicas, ya que, como se ha mencionado anteriormente, la mayoría de la producción de mandarinas se destina al consumo en fresco (Martínez-Ferrer et al. 2004; Aucejo-Romero 2005; Ansaloni et al. 2008). Por ello, unido a los costes de su gestión, *T. urticae* está considerada como una plaga clave en el cultivo de los cítricos.

Fig. 1.4. Daño en hoja.

Fig. 1.5. Daño en fruto.

1.2.4. Métodos de muestreos y umbrales de tratamientos

Los métodos de muestreo y los umbrales de tratamientos son herramientas fundamentales para la toma de decisiones relativas al control integrado de *T. urticae*. Los muestreos permiten valorar el daño provocado por la plaga y conocer cuándo ésta alcanza los umbrales preestablecidos. Sin embargo, antes de cualquier actuación, hay que tener en cuenta la proporción de *T. urticae* y sus enemigos naturales, principalmente los ácaros depredadores de la familia Phytoseiidae, a fin de priorizar la conservación de estos últimos. En este punto, la elección de un plaguicida selectivo frente a fitoseidos es clave en la gestión de esta plaga.

Para estimar las poblaciones de *T. urticae* en cítricos, se considera las hembras adultas, por su tamaño y porque constituyen una unidad aceptable para estimar la población total de formas móviles (Martínez-Ferrer et al. 2005; Martínez-Ferrer et al. 2006). La frecuencia de los muestreos depende de la incidencia de la plaga; cuando la densidad de *T. urticae* está lejos del UED, la frecuencia de muestreo puede ser más espaciada (quincenales). Sin embargo, cuando los síntomas comienzan a aparecer, los intervalos de muestreos deben ser menores (semanales). El muestreo se realiza lanzando dos aros de 56 cm de Ø (Fig. 1.6) sobre la copa de los árboles (en 18 – 32 árboles/hectárea) y contando el número de "aros ocupados", es decir, aquéllos que contienen dos o más hojas sintomáticas (manchas cloróticas) (Martínez-Ferrer et al. 2005; Martínez-Ferrer et al. 2006). Cuando el porcentaje de aros ocupados es menor de un 54%, no se requiere ninguna acción, porque no se sobrepasa el umbral económico de daños. En el caso que éste supere el 54%, se selecciona de cuatro a seis hojas sintomáticas al azar por aro y se determina el número hojas ocupadas por *T. urticae*, es decir, aquéllas que contienen al menos una hembra adulta. Si el porcentaje de hojas ocupadas es menor de un 22%, ninguna acción es requerida, porque no se sobrepasa el umbral establecido. Sin embargo, cuando la población de *T. urticae* supera estos umbrales, 54% de aros ocupados y/o 22% de hojas sintomáticas ocupados por *T. urticae*, se deben de tomar las medidas adecuadas para controlar las poblaciones de este fitófago. Este método de muestreo está relacionado con el umbral empírico establecido, que equivale a 0,48 ácaros por hoja sintomática (Martínez-Ferrer et al. 2005; 2006). Sin embargo, cabe resaltar que actualmente, Pascual-Ruiz y colaboradores de la Universitat Jaume I (UJI) (Castellón de la Plana, España), están desarrollando un nuevo umbral de económico de daños calculado de forma científica, para la toma de decisión con respecto a *T. urticae* y en presencia de los fitoseidos (J. Jacas comunicación personal).

Fig. 1.6. Aro utilizado para el muestreo de *T. urticae.*

1.2.5. Métodos de control

La reducción de las poblaciones de *T. urticae* requiere la utilización de diversos métodos o técnicas de control. Entre ellos destacan el control químico, el biológico y el cultural.

- *Control químico*:

El control químico sigue siendo el método más utilizado para controlar *T. urticae* tanto en campo como en viveros. Los tratamientos químicos deben hacerse siempre que la densidad de *T. urticae* sobrepase el UED, y no de manera preventiva o por observar sólo síntomas. Los ataques de este ácaro suelen aparecer en focos bien delimitados, por lo que es importante la vigilancia de éstos y, si es posible, realizar tratamientos localizados a estos focos antes de que se extiendan al resto del cultivo. En el caso particular de este fitófago es muy importante la alternancia entre materias activas con distintos modos de acción para evitar el desarrollo de resistencias en unas pocas generaciones, debido a su alta fecundidad y corto ciclo de vida. El control químico es una práctica que es eficaz a corto plazo, lo que a menudo lleva a los productores a repetir los tratamientos de manera inadecuada o incluso al uso de sustancias no autorizadas que pueden causar serios problemas de resistencia y/o eliminación de la fauna útil. En España, actualmente, las materias activas registradas y recomendadas para el control de *T. urticae* en cítricos están disponibles en la página web de los órganos competentes del Ministerio de Agricultura, Alimentación y del Medio Ambiente (GVA 2012; MAGRAMA 2012) (Tabla 1.1).

Table 1.1. Lista de materias activas actualmente registradas y recomendadas contra *T. urticae*.

Modo de acción	**Materia activa**	**Plazo de seguridad (días)**
Activador del canal cloro	Abamectina	10
Inhibidores de crecimiento de los ácaros	Clofentezin	21
	Hexitiazox	14
	Etoxazol	14
Inhibidores del transporte de electrones punto 1	Piridaben	15
	Tebufenpirad	7
	Fenpiroximato	14
Inhibidores de la síntesis ATP mitocondrial.	Propargita	14
Inhibidores de la síntesis de los lípidos	Spirodiclofen	14
Físico, asfixia.	Aceite mineral 79%	7

(GVA 2012; MAGRAMA 2012)

Las aplicaciones químicas deben realizarse a dosis recomendadas y sobre todo las técnicas de aplicación deben permitir alcanzar bien el envés de las hojas de manera que se asegure una apropiada cobertura vegetal. La aplicación correcta y eficaz de un acaricida para el control de *T. urticae* en cítricos exige un conocimiento completo de la biología de esta plaga.

En los últimos años, el control químico se está haciendo de manera más sostenible, dando prioridad a la conservación de los enemigos naturales, mediante la utilización de plaguicidas con menor impacto sobre ellos. El uso de plaguicidas selectivos combinados junto a tácticas de gestión de plagas como la conservación y/o sueltas aumentativas de ácaros depredadores, pueden ser estrategias importantes para el éxito de la GIP en cultivos de cítricos (Jacas & Urbaneja 2008; Urbaneja et al. 2008; Jacas & Urbaneja 2010)

- *Control cultural*:

El control cultural consiste en la utilización de técnicas o prácticas culturales determinadas con el propósito de contribuir a prevenir los ataques de las plagas, hacer el ambiente menos favorable para su desarrollo o disminuir sus daños. Algunos ejemplos de estas técnicas son: la rotación de cultivos, las técnicas de fertilización y manejo del riego, el uso de variedades resistentes, la poda, la cobertura del suelo, etc.

El correcto manejo del riego puede llevar a reducciones de los daños causados por *T. urticae*, teniendo en cuenta que los cítricos sometidos a estrés salino generalmente acumulan metabolitos como mecanismo de defensa (Arbona et al. 2003) y estos compuestos pueden afectar al desarrollo de los ácaros. En un estudio realizado por Aucejo-Romero et al. (2004) se demostró que bajas o moderadas concentraciones de NaCl en el agua de riego podrían ser la causa del aumento en la reproducción de *T.urticae* en clementinos. Ansaloni et al. (2008) también demostró que las prácticas culturales (sobre todo un programa de riego adecuado) y un buen control de las plagas que atacan las hojas nuevas (minador y pulgones) pueden favorecer la presencia de brotes tiernos en verano y otoño, y por lo tanto evitar que *T. urticae* migre a los frutos y cause daños. En cultivos perennes como los cítricos la cobertura del suelo puede ser importante para regular las poblaciones de ácaros, ya que puede darse la migración de la araña roja y los enemigos naturales de las malezas a los cultivos y viceversa (Barbosa 1998; Nyrop et al. 1998; Landis et al. 2000). Por ello, en los cítricos españoles, la práctica más difundida ha consistido en el mantenimiento de suelo desnudo mediante la aplicación de herbicidas (Gómez de Barreda 1994). Sin embargo, los herbicidas pueden causar algunos inconvenientes, entre ellos: migraciones masivas de *T. urticae* a la copa (Kim & Lee 2003; Hardman et al. 2005), efectos negativos en los enemigos naturales (Pratt & Croft 2000) y selección especies de plantas que hospedan poblaciones de araña roja (Aucejo-Romero et al. 2003).

- *Control biológico*:

Entre las distintas estrategias de control biológico de *T. urticae*, se está dando mucho énfasis en los últimos años al **control biológico por conservación** mediante la gestión de la cubierta vegetal y el **control biológico por inoculación** a través de las sueltas aumentativas de ácaros fitoseidos. Con respecto al control biológico por conservación mediante el manejo de la cubierta vegetal, se ha estudiado recientemente el uso de la gramínea *Festuca arundinacea* Schreber (Poaceae) en la regulación de las poblaciones de *T. urticae*. La festuca sembrada entre líneas del cultivo, actúa como un reservorio manteniendo las poblaciones de los enemigos naturales nativos o autóctonos (principalmente fitoseidos) elevadas durante todo el año. De esta manera, los depredadores actúan regulando y disminuyendo la presencia de *T. urticae* en los árboles (Aguilar-Fenollosa et al. 2011a). Por ello, el uso de esta cubierta vegetal está siendo ampliamente recomendado a los agricultores. La segunda estrategia de CB se refiere a las sueltas aumentativas de los ácaros fitoseidos *Neoseiulus californicus* (McGregor) y *Phytoseiulus persimilis* Athias-Henriot (Acari: Tetranychidae). El éxito de las sueltas de ambos depredadores se ha demostrado en ensayos de semi-campo con clementinos (Abad-Moyano et al. 2010a). En los campos de cítricos, estos ácaros depredadores son frecuentes, pero no son suficientemente abundantes. La aplicación de esta estrategia en condiciones de campo puede ser importante en la regulación de las poblaciones de *T. urticae* (Jacas & Urbaneja 2010).

El agroecosistema cítrico español alberga un variado complejo de enemigos naturales de *T. urticae*. Su identificación es un paso importante en la conservación y otras estrategias de gestión para la mejora del control biológico de *T. urticae*. En este sentido estudios anteriores han identificado numerosos enemigos naturales de esta plaga, pertenecientes a distintos grupos taxonómicos, cuya abundancia en el cultivo y eficacia en el control de *T. urticae* varía en función del cultivo y época del año (Tabla 1.2).

Table 1.2. Enemigos naturales de *T. urticae* descritos en cítricos en España.

Orden	**Familia**	**Especie**
Mesostigmata	Phytoseiidae	*Euseius stipulatus* (Athias-Henriot) *Phytoseiulus persimilis* Athias-Henriot *Neoseiulus californicus* (McGregor) *Typhlodromus phialatus* (Athias-Henriot)
Coleoptera	Coccinellidae	*Stethorus punctillum* (Weise) *Scymnus interruptus* (Goeze) *Scymnus mediterraneus* (Lablokoff'khnzor)
Neuroptera	Chrysopidae	*Chrysoperla carnea* (Stephens)
	Coniopterygidae	*Conwentzia psociformis* (Curt)
Thysanoptera	Thripidae	*Semidalis aleyrodiformis* (Stephens) *Scolothrips longicornis* (Priesner) *Scolothrips sexmaculatus* (Pergande) *Aelothrips intermedius* (Bagnall)
Diptera	Cecidomyiidae	*Feltiella acarisuga* (Vallot)
Hemiptera	Reduviidae	*Empicoris rubromaculatus* (Blackburn)

(Ripollés & Melia 1980; García-Marí et al. 1991; Lacasa & Llorens 1998; Alvis 2003; Abad-Moyano et al. 2008; Abad-Moyano et al. 2009a)

1.3. Ácaros depredadores

Los principales enemigos naturales de *T. urticae* pertenecen al grupo de los ácaros depredadores. Los ácaros depredadores han sido importantes agentes de control biológico, desde finales de los años ‘60 e inicios de los ‘70, constituyendo elementos esenciales en algunos programas de gestión de plagas (McMurtry & Croft 1997). Son varias las familias de ácaros depredadores, entre ellas se puede citar: la familia Phytoseiidae, Stigmaeidae, Hemisarcoptidae, Trombidiidae, Cheyletidae, Erythraeidae, Anystidae, Tydeidae, etc. (García-Marí et al. 1991; Abad-Moyano et al. 2008).

1.3.1. Phytoseiidae

La familia Phytoseiidae destaca por su abundancia en los cultivos y su eficiencia como depredadores de ácaros fitófagos. Se trata de una familia de amplia distribución mundial con más de 2000 especies descritas pertenecientes a 67 géneros distintos en todo el mundo. Los ácaros de la familia Phytoseiidae han resultado ser excelentes agentes de control biológico y por ello tienen gran importancia desde el punto de vista agrícola (Helle & Sabelis 1985b; Croft 1990; McMurtry 1992) formando parte de muchos programas de gestión de ácaros fitófagos y de trips, tanto en invernaderos como en plantaciones al aire libre, diversos cultivos ornamentales, vegetales, granos y frutales de todo el mundo (Moraes & Flechtmann 2008).

Los fitoseidos son ligeramente más grandes que los ácaros fitófagos, con aproximadamente 0,5-0,8 mm de largo, son de movimientos rápidos, y buscan activamente a sus presas. Su coloración suele ser blanquecina con aspecto brillante. Tienen un ciclo de vida corto, con un promedio de 6-7 días, dependiendo de las condiciones ambientales. Los fitoseidos difieren en sus estilos de vida en función de su régimen alimenticio, ya que diferentes especies tienen preferencia por distintos tipos de alimentos presas: como pequeños artrópodos (ácaros, trips, moscas blancas), hongos, polen y exudados vegetales (Helle & Sabelis 1985b; McMurtry & Croft 1997).

McMurtry y Croft (1997) clasificaron los fitoseidos en cuatro grandes grupos: el grupo I, representado por los depredadores especializados en *Tetranychus* spp., que incluye el género *Phytoseiulus*; el grupo II, compuesto por depredadores selectivos de ácaros tetraníquidos que producen densas telarañas, representados por *Galendromus* spp. y algunas especies del género *Neoseiulus*. Las especies pertenecientes a este grupo también pueden alimentarse de otros tipos de ácaros, y por tanto no son tan especializados como las especies del grupo I; el grupo III, compuesto por depredadores generalistas que se alimentan de diversas fuentes de alimentos como ácaros, polen e insectos como moscas blancas, thrips y cochinillas. Este grupo está representado principalmente por los géneros *Amblyseius* spp., *Typhlodromus* spp.; y el grupo IV, representados por depredadores generalistas, especialistas en polen, tales como los fitoseidos del género *Euseius* (McMurtry & Croft 1997; Croft et al. 2004). Así, algunas especies de fitoseidos pueden desarrollarse y reproducirse, alimentándose de fuentes alternativas como polen y melaza. En estudios recientes se ha observado que la calidad del polen presente en el sistema cítrico puede favorecer la abundancia de algunas especies, más que otras. En este sentido, en los últimos años se ha dado mucho énfasis al uso del polen como fuente de alimento para el establecimiento y mantenimiento de poblaciones de fitoseidos en agroecosistemas de cara a regular las poblaciones de fitófagos (Ferragut et al. 1987; Zhimo & McMurtry 1990; Grafton-Cardwell et al. 1999; Nomikou et al. 2002; Ragusa et al. 2009). Sin embargo, la presencia de polen puede resultar perjudicial para el control, en el caso en el que la plaga también se alimenta de polen, por ejemplo los trips fitófagos (van Rijn et al. 2002).

1.3.2. Phytoseiidae en cítricos

En los cítricos españoles se han identificado 12 especies de fitoseidos (García-Marí et al. 1986; Abad-Moyano et al. 2009a), al menos 8 de estas especies se han descrito en la cubierta vegetal (Aucejo-Romero 2005; Aguilar-Fenollosa et al. 2011a). Las especies más importantes y comúnmente encontradas en las copas de los huertos de clementinos asociadas a colonias de *T. urticae* y/o *Panonychus citri* (McGregor) (Acari: Tetranychidae) son: *Euseius stipulatus* (Athias-Henriot), *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) y *Thyphlodromus phialatus* (Athias-Henriot) (Abad-Moyano et al. 2009a).

1.3.2.1. *Euseius stipulatus* (Athias-Henriot)

Es el ácaro fitoseido más abundante en los cítricos españoles. Puede encontrarse tanto en el árbol como en la cubierta vegetal (García-Marí et al. 1991; Aucejo et al. 2003; Abad-Moyano et al. 2009a; Aguilar-Fenollosa et al. 2011a). *Euseius stipulatus* (Fig. 1.7) es un depredador generalista especializado en polen (McMurtry & Croft 1997). Este fitoseido juega un papel importante en el control natural del ácaro rojo *P. citri*. En cambio, *E. stipulatus* no es un buen agente de control de *T. urticae*, debido a la baja adecuación a este alimento y la dificultad para entrar en la tela de esta araña. En estudios de laboratorio, sobre hojas de cítricos, Abad-Moyano et al. (2009b) y Ferragut et al. (1987) observaron que la tasa de puesta y el tiempo de desarrollo de *E. stipulatus* se veía afectado negativamente cuando era obligado a alimentarse con *T. urticae*.

1.3.2.2. *Neoseiulus californicus* (McGregor)

Se considera un depredador polífago especializado en ácaros tetraníquidos (McMurtry & Croft 1997) pero también puede alimentarse y sobrevivir con otras fuentes de alimento, como el polen. *Neoseiulus californicus* (Fig. 1.8) es uno de los fitoseidos más frecuentes en los cítricos españoles y suele encontrarse asociados a los ácaros tetraníquidos. Este depredador ha demostrado ser un buen agente de control de los ácaros de la familia Tetranychidae incluyendo *P. citri* (McMurtry 1977; García-Marí et al. 1986; Katayama et al. 2006), *Panonychus ulmi* (Costa-Comelles et al. 1986), *T. urticae*, *Tetranychus turkestani* y *Tetranychus ludeni* (Costa-Comelles et al. 1986; Escudero & Ferragut 2005).

En los cítricos españoles, *N. californicus* suele alcanzar altas densidades sólo cuando hay elevada densidad de presa. Por lo contrario, cuando la presa es escasa o ausente, éste vuelve a disminuir o no se establece satisfactoriamente en el cultivo (García-Marí et al. 1986). Por lo tanto, sueltas aumentativas de este fitoseido o combinadas con otras especies pueden ser adecuadas para el control de ácaros plagas (Jacas & Urbaneja 2008). Entre las cualidades favorables de *N. californicus* destacan, su excelente tolerancia a las altas temperaturas, la baja humedad y a algunos pesticidas (Castagnoli & Simoni 1991; Castagnoli & Simoni 2003; Walzer et al. 2007; Palevsky et al. 2008). En estudios recientes, se observó que *N. californicus* juega un papel importante en estrategias de control biológico por inoculación, reducciendo la infestación de la araña roja *T. urticae* en condiciones de semi-campo de cítricos (Abad-Moyano et al. 2009a)

1.3.2.3. *Phytoseiulus persimilis* Athias-Henriot

Es un depredador especializado en ácaros del genero Tetranychus, y su área de distribución se limita a zonas húmedas. *Phytoseiulus persimilis* (Fig. 1.9) es el fitoseido más conocido y empleado en el control de tetraníquidos de numerosos cultivos (García-Marí et al. 1991;

McMurtry & Croft 1997). En los cítricos españoles, *P. persimilis* es poco abundante (García-Marí et al. 1986; Abad-Moyano et al. 2009a), pero es conocido por ser el depredador más voraz de *T. urticae* en los cítricos australianos (Smith et al. 1997). Este depredador tiene un ciclo de desarrollo rápido, superior al de su presa en condiciones normales, y su fecundidad y capacidad de consumo de presas es la más alta de las encontradas en fitoseidos (Badii & McMurtry 1984; Gotoh et al. 2004; Escudero & Ferragut 2005; Popov & Khudyakova 2008). *Phytoseiulus persimilis* mantiene un control efectivo con temperaturas entre 15° y 25°C en intervalos de 60-90% de humedad relativa. No tolera bien las altas temperaturas (> 30°C), y baja humedad relativa (< 60%) (Hamamura et al. 1976; Sabelis 1981).

Fig. 1.7. Hembra de *E. stipulatus*

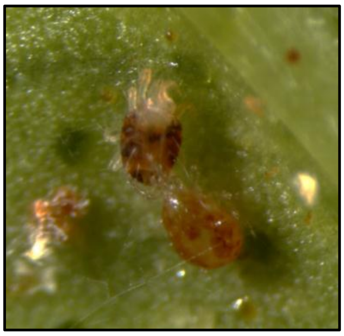

Fig. 1.8. Hembra de *N. californicus* depredando *T. urticae*

Fig. 1.9. Inmaduro de *P. persimilis*

1.4. Objetivos

Hasta ahora, el control de *T. urticae* en huertos de clementino se ha basado principalmente en el uso de acaricidas. Sin embargo, como se ha mencionado anteriormente, este método de control implica una serie de inconvenientes. Por lo tanto, es necesario encontrar formas alternativas, ambientalmente seguras para controlar esta plaga, como por ejemplo, el control biológico. En este contexto, el objetivo global de esta tesis doctoral ha sido la **mejora del Control Biológico (CB) de *T. urticae* en clementinos.**

El CB es un componente importante de la gestión integrada de plagas (GIP). La conservación y las sueltas aumentativas de enemigos naturales se incluyen dentro de las estrategias de gestión de plagas en cítricos, tanto en campo como en viveros. Recientemente, las liberaciones inoculativas de los fitoseidos *P. persimilis* y *N. californicus* en condiciones de semi-campo han dado resultados altamente satisfactorios en el control de *T. urticae*. Sin embargo, de acuerdo con las prácticas actuales de gestión en viveros, la implementación de ambas estrategias resulta dificil, principalmente debido a los efectos secundarios de la gran cantidad de tratamientos químicos dirigidos a otros fitófagos. La elección de los plaguicidas selectivos que sean compatibles con sus enemigos naturales sería una alternativa para mejorar la gestión de *T. urticae* tanto en campo como en viveros cítricos. Como primer paso, se ha estudiado **la compatibilidad de *Phytoseiulus persimilis* y *Neoseiulus californicus* con el neonicotenoide imidacloprid para la gestión de plagas en viveros de clementinos** (Capítulo 2). En segundo lugar, disponer de mayor información sobre la selectividad de otros plaguicidas utilizados cítricos sobre los tres ácaros depredadores más importantes nos ayudaría a identificar si alguno de ellos podría ser utilizado como especie indicadora de los efectos secundarios de plaguicidas en este cultivo. Esta información podría ser útil para refinar las opciones de gestión contra los ácaros plaga. Por ello, el segundo objetivo fue el estudio de los **efectos secundarios de diferentes plaguicidas sobre *Euseius stipulatus*, *P. persimilis* y *N. californicus*** (Capítulo 3).

El establecimiento y mantenimiento de las poblaciones de fitoseidos en el sistema puede mejorar la regulación de *T. urticae* en clementinos. Sin embargo, en periodos de baja densidad de *T. urticae*, las poblaciones de ácaros depredadores suelen reducirse, y en particular en el caso de depredadores especializados en tetraníquidos. Por tanto, la disponibilidad de fuentes de alimentos alternativos no-presa, como el polen, puede favorecer el mantenimiento de los ácaros depredadores omnívoros en el cultivo y regular las poblaciones de *T. urticae*. Por esta razón, el tercer objetivo se centra en el estudio del **papel del polen en el CB de *T. urticae* en mandarinos clementinos** (Capítulo 4).

Las sueltas aumentativas de *N. californicus* y *P. persimilis* para el control de *T. urticae* se ha probado exitosamente en viveros de cítricos. Sin embargo, las sueltas que se han hecho en condiciones de campo, no han dado resultados consistentes. Especialmente, en el caso de *N. californicus* que en ocasiones ha llegado a controlar a la araña, pero sin poder ser recuperado después en el árbol. Prácticamente no existe información sobre el comportamiento de estos fitoseidos en los árboles de cítricos tras las sueltas. Por esta razón y para estar en condiciones de poner a punto un método de muestreo y un tipo de suelta más acertado, el cuarto objetivo se centró en el estudio de la **distribución espacial de los fitoseidos *P. persimilis* y *N. californicus* en plantas jóvenes de clementinos** (Capítulo 5).

CAPÍTULO 2

COMPATIBILITY OF *Phytoseiulus persimilis* AND *Neoseiulus californicus* (ACARI: PHYTOSEIIDAE) WITH IMIDACLOPRID TO MANAGE CLEMENTINE NURSERY PESTS

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2.1. Abstract

Biological control is not common in citrus nurseries where chemical control is prevalent. The systemic neonicotinoid imidacloprid applied as a drench is effective against three out of four key pests of young clementine plants in Spain - aphids, leafminers, and scales. However, mites, the fourth key pest, are not controlled by imidacloprid and could be regulated by introduction of the predatory phytoseiid mites *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor). The aim of this study was to evaluate the effects of imidacloprid applied as a drench on the demographic parameters of these two predatory mites and the compatibility of *P. persimilis* releases with imidacloprid to control key pest populations in young clementine plants under field conditions. The results showed that some demographic parameters of *P. persimilis* were affected by imidacloprid. However, their combined effect on its intrinsic rate of increase was neutral. In contrast, imidacloprid negatively affected the demographic parameters of *N. californicus*. Field results proved that young clementine plants could be satisfactorily protected against key pests with releases of *P. persimilis* combined with drench applications of imidacloprid. The combination of imidacloprid with *P. persimilis* releases was highly effective for management of the key pests of young clementine plants in the nursery.

Key words: Phytoseiids; Citrus; Chemical treatment; demographic parameters.

2.2. Introduction

Orchard and nursery citrus trees share most pest species. Nevertheless, their economic importance in each system differs considerably. Key pests of citrus orchards in Spain are those directly damaging the fruit, such as California red scale, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) and Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) (Jacas & Urbaneja 2010). However, arthropods affecting new foliage, such as the aphids *Aphis spiraecola* Pagenstecher and *A. gossypii* Glover (Hemiptera: Aphidae), the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and the spider mites *Panonychus citri* (McGregor) and *Tetranychus urticae* Koch (Acari: Tetranychidae), are considered key pests in nurseries.

Management strategies in Spanish citrus orchards are based on the conservation of natural enemies (Jacas & Urbaneja 2010). This strategy though is not applicable to the ephemeral nursery system, where chemical control is still the preferred method. Up to 20 chemical treatments per year can be applied in Spanish citrus nurseries (F. Llatser AVASA, personal communication). This is a costly strategy both for nurserymen and the environment, which can cause serious problems of (1) efficacy, (2) resistance (as in the cases of aphids and *T. urticae*), (3) side effects on workers and non-target species and (4) sustainability. As a consequence of the Regulation of Plant Protection Products in Europe under Council Directive 2009/128/EC (UE 2009), there is a need for more sustainable and efficient alternative control methods against citrus nursery pests. For this reason, identification of pesticides selective to candidate invertebrate biological control agents for use against nursery pests is a prerequisite for the introduction of biological control strategies in nurseries.

Recently, inoculative releases of the predatory mites *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) have been successfully used to control *T. urticae* in clementine young plants (Abad-Moyano et al. 2010a). However, mite biological control with phytoseiid releases is not possible under the current management practices in nurseries, mainly due to unacceptable side-effects of the pesticides targeted against aphids and citrus leafminer on predatory mites (Zhang & Sanderson 1990; Van de Viere & Tirry 2003; Bostanian & Akalach 2006; Urbaneja et al. 2012).

In Spain, there are about 20 active substances authorized against either aphids or the citrus leafminer (MAGRAMA 2012) and from these, 12 are recommended under an integrated production label (Urbaneja et al. 2012). Although some insecticides permitted for aphid control (pirimicarb, pymetrozine and azadirachtin), are considered harmless to slightly harmful for predatory mites (James 2002; Duso et al. 2008), control of the citrus leafminer in nurseries relies abamectin which is categorized as harmful to phytoseiids (Van de Viere & Tirry 2003). The systemic neonicotinoid imidacloprid applied by drench is also authorized for use against the citrus leafminer, and this type of application is also effective on sucking hemipteran pests, such as aphids, whiteflies and scales (Powell et al. 2006). This insecticide is considered harmless to *T. urticae* (James & Price 2002; Anh et al. 2004; Ako et al. 2006) and its effects on phytoseiid mites are disputed. While some authors consider imidacloprid to be harmless for phytoseiids (Mizell & Sconyers 1992; James 1997; James & Vogele 2001; Kim et al. 2005). Poletti et al (2007) described changes in the functional response of *N. californicus* and *Phytoseiulus macropilis* (Banks), with reductions of 55% and 87% in peak prey consumption for these species, respectively, when fed imidacloprid-treated prey. Similar results have been obtained by other authors (James 2003; Villanueva & Walgenbach 2005; Bostanian et al. 2009; Bostanian et al. 2010), but completely opposite effects have also been described (James 1997; Castagnoli et al. 2005). Because the combined use of predatory mites and imidacloprid could offer an interesting alternative for the management of citrus nursery pests, the aims of this study have been to firstly evaluate the effect of imidacloprid when applied as a drench on the demographic parameters of *P. persimilis* and *N. californicus* under laboratory conditions, and secondly to check the compatibility of imidacloprid and the release of these predatory mites in successfully managing citrus nursery pests under field conditions.

2.3. Materials and Methods

2.3.1. Laboratory assay

2.3.1.1. Spider mite stock colonies

The initial colony of *T. urticae* was obtained from mites collected in clementine orchards in the region of La Plana (Castelló, Spain). This colony, which was later used in our assays, was maintained on lemon fruits in a climatic chamber at 25 ± 1°C, 70-80% RH and 16:8 h (L:D) photoperiod. Lemons were collected from a pesticide-free orchard located at the Valencian Institute of Agricultural Research (IVIA). Fruits were brushed under water and dried with absorbent paper to remove any arthropods before being placed in the rearing units. Fruits were placed on a wooden frame (26 × 10 × 7 cm) two of which were positioned inside a plastic tray (45 × 30 × 8 cm) filled with water and soap to prevent mites to prevent escape and contamination with other mite species. Five new lemons were placed weekly in the *T. urticae* rearing unit with each set of five lemons left inside the rearing unit for 21 days. Thus, there were always 15 infested lemons in each rearing unit.

2.3.1.2. Predatory mite stock colonies

Colonies of *P. persimilis* and *N. californicus* were initiated with individuals from commercial mass rearing (SPIDEX® and SPICAL®, respectively, Koppert Biological Systems, SL., Águilas, Spain). Rearing units were similar to those described by Overmeer (1985): a piece of hard black plastic was placed over a water saturated sponge. The sponge with the plastic piece was stood in a plastic tray with water. The plastic borders were covered with thin sheets of tissue paper to ensure a constant water supply to the phytoseiids, to fix the plastic piece to the sponge and to prevent phytoseiids from escaping. Twice a week, *T. urticae* infested clementine leaves were added to each phytoseiid rearing unit. To avoid rearing contamination, each species was kept in different climatic chambers at the same conditions described above for *T. urticae*.

2.3.1.3. Plant material and infestation

Two-year-old clementine plants (*Citrus reticulata* Blanco [cultivar Clementina de Nules] grafted onto 'Carrizo' citrange rootstock [*Poncirus trifoliata* (L.) Rafinesque-Schmaltz x *Citrus sinensis* (L.) Osbeck were used in our assays. A batch of 24 plants was pruned, defoliated and immediately treated with 1.5% mineral oil (Sunspray Ultra-Fine® 85% p/v, Agrichem S.A., Madrid, Spain). Plants were held in a greenhouse located at IVIA maintained at 22 ± 2°C, 55 ± 10% RH and natural photoperiod and watered three times a week. Plants were ready for assay one month after defoliation, when they had around 150 young fully-expanded leaves. At this time half of the plants were each drenched with 500 ml of a solution containing 1 ml of imidacloprid (Confidor 20 OD®, (20% w/v active substance) Bayer Crop Science). Previous assays showed that 500 ml were enough to wet the plant surface without leaking from the pot. One week later, each of eight plants per treatment were infested with 10 *T. urticae* adult females (obtained from the lemon colony) deposited on the upper side of different randomly-chosen leaves. Four infested plants from each treatment were used as a source of *T. urticae* placed in experimental arenas (describe later) using leaves collected from the remaining four uninfested plants from each treatment. The whole process was repeated at 15 day intervals to ensure regular provision of both clementine leaves and mites (control and imidacloprid-treated) for the experiment.

2.3.1.4. Experimental unit (arena)

The experimental unit was a modified Huffaker cell (Sabelis 1981; Overmeer 1985). This unit consisted of a clear acrylic bottom plate (80 × 40 × 5 mm) and a top acrylic plate (80 × 40 × 10 mm). The top plate had a central circular hole (Ø 2 cm). On each lower plate, a clementine leaf from either an imidacloprid-treated or a control plant was placed upside down on top of a piece of filter paper (80 × 40 mm) in such a way that the leaf substrate formed the bottom of the inner side of the arena. The upper plate was sealed to the leaf with plasticine (Plastilina Jovi® JOVI, S.A. Barcelona, Spain) and both plates were held together with two rubber bands. The hole in the upper plate was closed with a glass cover slip (40 × 35 mm) held in place with transparent adhesive tape.

Five *T. urticae* females were introduced into each cell (respectively, control females reared on untreated clementine leaves or those reared on imidacloprid-treated clementine plants) and allowed to settle, feed and oviposit for 5 days prior to the introduction of phytoseiids. Hence, the phytoseiid mite subsequently introduced into the cell (see below) had different *T. urticae* stages to prey upon and some webbing for oviposition sites. After the introduction of the phytoseiids, an ample supply of all spider mite stages from either treated or control plants was provided. Arenas were maintained in a climatic chamber at 25 ± 1 °C, 80 ± 5% RH and 16:8 h (L:D) photoperiod during the whole experiment.

To confirm that imidacloprid was present in the treated plants, 20 treated and 20 uninfested control leaves were randomly collected from the clementine plants 3 days before the onset of the assays. Each leaf was enclosed in a modified Huffaker cell as above. Subsequently, 10 *Aphis gossypii* Glover (Hemiptera: Aphididae) adults obtained from a stock colony established at IVIA (De Mendoza et al., 2006) were deposited on each arena, and mortality scored 24 hours later (Nauen et al. 1998). All the plants could be used to the assay, since 100% aphid mortality in treated leaves and less than 15% in control plants was fulfilled.

2.3.1.5. Development time

Two separate assays, one with *P. persimilis* and another one with *N. californicus* were carried out. For each species, 120 mated females were individually isolated in the arenas described above, 60 of them on treated leaves and the remaining 60 on control leaves. The arenas were frequently checked under a binocular microscope and once oviposition had taken place, females were removed, leaving only one egg per arena. Afterwards, hatching, molting, and survival were checked every eight hours until adulthood or death. Presence of exuvia was used as evidence of molting. An ample supply of *T. urticae* (all stages) from control or treated plants, for each respective treatment, was provided as needed.

2.3.1.6. Reproductive parameters

Once female phytoseiids reached the adult stage, a young male obtained from the stock colony was added to the arena. Couples were observed every eight hours until first oviposition. Afterwards, couples were transferred to new arenas every 24 h during five days, and the number of eggs laid per female/arena/day was counted (Janssen & Sabelis 1992; Abad-Moyano et al. 2009b). The progeny were fed with untreated *T. urticae* grown on bean leaves until they reached the adult stage. Then, they were slide-mounted for sex determination. Mean pre-oviposition time, fertility, oviposition rate and sex ratio were calculated for each female.

2.3.1.7. Demographic parameters

Estimates of the intrinsic rates of increase (r_m) of the phytoseiids were obtained for each treatment by the Methuselah reproduction schedule version which assumes that mites live indefinitely and reproduce at their peak rate (Janssen & Sabelis 1992). Although this method overestimates r_m due to the simplified life-history schedule (no mortality and no age-dependent reproduction), these estimates are close to those obtained from full life-table analysis (Janssen & Sabelis 1992). Intrinsic rate of increase was calculated by iteratively solving the following equation:

$$r_m = R_0(T) \cdot \mathrm{exp}^{[(-r_m) \cdot A]}$$

where R_0 = net reproductive rate; T = age at peak oviposition, and A = age at first oviposition. The net reproductive rate at peak oviposition ($R_0(T)$) was calculated as:

$R_0(T)$ = (peak oviposition rate) x (survival egg-to-egg period) x (sex ratio)

Peak oviposition rates were calculated from the average female oviposition rates between the 2nd and 5th day. The Jackknife procedure was used to estimate a standard error of the $R_0(T)$ and r_m values. Subsequently, different $R_0(T)$ and r_m values were compared using *t*-tests.

2.3.2. Field assay

2.3.2.1. Study site and plant material

Forty-eight 2-year-old clementine plants, pruned and treated as explained before or the laboratory assay, were transferred to a cleared 400 m^2 area close to citrus orchards and vegetable crops at IVIA experimental fields (Moncada, Spain). Plants were arranged in a complete randomized block design of 4 x 3 groups of 4 plants (= replicates) and were watered from one to three times per week depending on the weather. A randomized block design including three different treatments was used. The study was conducted from 3 April to 3 December 2008.

2.3.2.2. Experimental procedure

Three treatments were evaluated: (A) an untreated control, (B) an IPM treatment using a combination of chemical and biological control and (Combined-IPM), and (C) an IPM treatment using chemical control only (Chemical-IPM).

The combined-IPM treatment consisted of applying imidacloprid as a drench (Table 2.1) and predatory mite releases. Imidacloprid was preventively applied in spring against aphids and at the beginning of the summer against *P. citrella* (Table 2.1), and releases of *P. persimilis* against *T. urticae* were based on action thresholds (see below). *Phytoseiulus persimilis* predatory mites (SPIDEX®) were purchased from Koppert Biological Systems, S.L., Águilas, Murcia, Spain and were released on 10 June and 2, 9 and 16 September 2008. Each release consisted of approximately 70 individuals per plant.

The Chemical-IPM treatment was based on decisions to intervene on sampling and action thresholds against aphids, *P. citrella* and *T. urticae* (see below). When necessary, pesticides were applied. Pesticides included in this evaluation, active substances, application dates, concentrations used, and information about the authorized dose in citrus (MAGRAMA 2012) are given in Table 2.1.

Plants were sampled on a weekly basis. Sampling consisted on throwing a ring 20 cm in diameter, made of flexible polyethylene irrigation tube, at a random location in the canopy of each plant. The numbers of total leaf flushes, and that of both aphid- and *P. citrella*-infested leaf flushes within the ring were recorded (adapted from De Mendoza et al. (2006), Karamaouna et al. (2010) and Urbaneja et al. (2000). Further, the number of *T. urticae*-occupied rings (those containing at least two symptomatic leaves) was scored. When present, two symptomatic leaves were randomly selected and the number of *T. urticae* adult females per leaf was counted (adapted from Martínez-Ferrer et al. (2004). Finally, the number of plants upon which *P. persimilis* could be found was scored. Action thresholds considered were 25 % occupied rings for aphids (De Mendoza et al. 2006), more than 1 infested leaf flush for *P. citrella* (Ripollés et al. 1996), and 54% and 22% of *T. urticae*-occupied rings and leaves, respectively (Martínez-Ferrer et al. 2004).

Table 2.1. Products used in the assays (MAGRAMA 2012).

	Application date (2008)	**Active ingredient**	**Trade name**	**Manufacturer**	**Max. authorized dose in citrus**[a]
Combined-IPM treatment	3 Apr, 15 May, 24 Jun	Imidacloprid (200 g l^{-1} OD)	Confidor©	Bayer	50-75 ml hl^{-1}
Chemical-IPM treatment	30 May	Pymetrozine (250 g l^{-1} WP)*	Plenum©	Syngenta	40g hl^{-1}
	2 Jul	Etoxazole (110 g l^{-1} SC)*	Borneo©	Kenogard	12.5-50ml hl^{-1}
	3 Jun	Pyridaben (200 g l^{-1} WP)*	Sanmite©	BASF	50-100 ml hl^{-1}
	24 Jul, 12 Aug	Abamectin (18 g l^{-1} EC)*	Abamectin©	Spanish Chemicals	50-100 ml hl^{-1}

*Approximately 200 ml of the formulated product were sprayed on each tree.

2.3.3. Data analysis

Mean development time, pre-oviposition time, peak oviposition rate, fertility and sex ratio were compared between treatments for each species using Student's t-tests ($P = 0.05$).

To compare the three management strategies (control, combined IPM and chemical IPM) the number of *T. urticae* occupied rings and that of *P. citrella*-infested flushes during the field assay were plotted against time. Likewise, we plotted the percentage of symptomatic leaves occupied by *T. urticae* and that of aphid-infested flushes. The area under the curves and above their respective action thresholds was estimated using the middle sum method of the Riemann technique (Apostol 1974). These values were considered as representative of damage and were subjected to one-way analyses of variance (*P* = 0.05). If necessary the Tukey's test for mean separation was applied.

2.4. Results

2.4.1. Laboratory assays

2.4.1.1. Development time

There were no significant differences for egg, larva and protonymph development times between imidacloprid and control treatments for either predatory mite species (Table 2.2). Deutonymph development time was significantly longer for *P. persimilis* (152.3% increase) and *N. californicus* (27.5% increase) when fed on *T. urticae* reared on imidacloprid treated clementine leaves in comparison to control treatment (Table 2.2). Overall, egg to adult development time was significantly longer in the case of *P. persimilis* only (7.1% increase) whereas no differences were observed for *N. californicus*.

Table 2.2. Mean development time ± SE in days of *P. persimilis* and *N. californicus* fed on *T. urticae* reared on clementine leaves with and without Imidacloprid at 25˚C.

	Egg	**Larva**	**Protonymph**	**Deutonymph**	**Egg to adult**
P. persimilis					
Control	1.57 ± 0.07 a n= 51	0.66 ± 0.06 a n= 51	2.02 ± 0.06 a n= 51	0.44 ± 0.07 b n= 51	4.52 ± 0.07 b n= 51
Imidacloprid	1.72 ± 0.05 a n= 49	0.71 ± 0.07 a n= 49	1.77 ± 0.16 a n= 49	1.11 ± 0.14 a n= 49	4.84 ± 0.13 a n= 49
(*t*; *df*; *P*)	1.58; 98; 0.117	0.54; 83; 0.592	-1.68; 38; 0.100	4.11; 41; <0.001	2.00; 89; 0.048
N. californicus					
Control	1.78 ± 0.04 a n= 54	0.66 ± 0.04 a n= 52	1.36 ± 0.05 a n= 57	0.91 ± 0.05 b n= 56	4.76 ± 0.06 a n= 54
Imidacloprid	1.72 ± 0.05 a n= 53	0.69 ± 0.05 a n= 47	1.34 ± 0.10 a n= 48	1.16 ± 0.08 a n= 44	4.97 ± 0.12 a n= 41
(*t*; *df*; *P*)	-1.20; 105; 0.231	-0.57; 97; 0.573	0.26; 103; 0.799	-2.54; 98; 0.013	-1.76; 93; 0.083

For each predatory mite, means in the same column followed by the same letter are not significant different (Student's *t*-test *P* = 0.05).

2.4.1.2. Reproductive parameters

Imidacloprid treatment had no effect on the duration of the pre-oviposition period for either predatory mite species (Table 2.3). Effects of imidacloprid on other reproductive parameters depended on the species. Imidacloprid-exposed *P. persimilis* showed a higher peak oviposition rate (4.29 *vs*. 3.00 eggs) and reduced egg hatch (84.9 *vs*. 98.4%), whereas sex ratio was independent of treatment. Sex ratio was significantly lower for imidacloprid-exposed *N. californicus* (57.1 *vs*. 69.1%). For both predatory mites, survival to adulthood was significantly reduced for the imidacloprid treatment, 13.8% in the case of *P. persimilis* and 40.4% for *N. californicus*.

Table 2.3. Pre-oviposition time, peak oviposition rate, egg hatching, sex ratio (means ± SE) and egg-to-egg survival (%) of *P. persimilis* and *N. californicus* fed on *T. urticae* reared on clementine leaves with and without Imidacloprid at 25˚C.

	Pre-oviposition time (days)	**Peak oviposition rate (eggs/♀ and day)**	**Egg hatch (%)**	**Sex ratio (♀/(♀ +♂))**	**Survival in egg-to-egg period (%)**
P. persimilis					
Control	1.28 ± 0.11 a	3.00 ± 0.24 b	98.35 ±1.111 a	62.78 ± 2.45 a	98.4 ± 0.95
	n= 18	n= 18	n= 18	n= 18	
Imidacloprid	1.14 ± 0.14 a	4.29 ± 0.29 a	84.85 ±3.77 b	51.68 ± 3.25 a	84.8 ± 2.64
	n= 7	n= 7	n= 7	n= 7	
(*t*; *df*; *P*)	0.68; 23; 0.499	-2.99; 23; 0.006	6.22; 23; <0.001	-0.92; 23; 0.369	6.22; 23;<0.001
N. californicus					
Control	1.88 ± 0.08 a	4.18 ± 0.18 a	98.71 ±1.14 a	69.06 ± 3.10 a	93.76 ± 0.86
	n= 17	n= 17	n= 17	n= 17	
Imidacloprid	1.84 ± 0.12 a	4.26 ± 0.15 a	96.26 ± 1.43 a	57.10 ± 4.72 b	55.84 ± 0.97
	n= 19	n= 19	n=19	n= 19	
(*t*; *df*; *P*)	0.28; 34; 0.781	-0.38; 34; 0.709	1.26; 34; 0.222	2.07; 34; 0.047	29.0; 34; <0.001

For each predatory mite, means in the same column followed by the same letter are not significant different (Student's *t*-test *P* = 0.05).

2.4.1.3. Demographic parameters

Peak oviposition $R_0(T)$ of imidacloprid-exposed specimens was significantly different compared with control for both species (Table 2.4). However, these differences were positive in the case of *P. persimilis* (15.7% increase) and negative in the case of *N. californicus* (49.7% decrease). In the case of the intrinsic rate of increase (r_m) differences were observed in *N. californicus* only (25.9% decrease).

Table 2.4. Jackknife estimates (means ± SE) of net reproductive rate at peak oviposition $R_o(T)$ and intrinsic rate of increase (r_m) for *P. persimilis* and *N. californicus* fed on *T. urticae* reared on clementine leaves with and without Imidacloprid at 25˚C .

	$R_o(T)$ (♀/♀)	**r_m**
P. persimilis		
Control	1.845 ± 0.013 b (n= 18)	0.302 ± 0.011 a (n= 18)
Imidacloprid	2.137 ± 0.028 a (n= 7)	0.316 ± 0.011 a (n= 7)
Statistics (*t*; *df*; *P*)	12.57; 23; <0.0001	-0.70; 23; 0.4907
N. californicus		
Control	2.703 ± 0.01 a (n= 17)	0.321 ± 0.01 a (n= 17)
Imidacloprid	1.359 ± 0.01 b (n= 19)	0.238 ± 0.02 b (n= 19)
Statistics (*t*; *df*; *P*)	106.4; 34; <0.0001	4.84; 34; <0.0001

For each predatory mite, means in the same column followed by the same letter are not significant different (Student's *t*-test *P* = 0.05).

2.4.2. Field assay

Tetranychus urticae repeatedly exceeded the action thresholds in all management strategies tested (Figs. 2.1 and 2.2). Therefore, *P. persimilis* was released four times in the plants subjected to the combined-IPM, and acaricides were applied to chemical-IPM plants (Table 2.1). As a consequence, the areas under the curve and above the threshold were significantly smaller in both IPM strategies than in the control (Table 2.5). Interestingly, in late September, *P. persimilis* was found in control plants where it had not been previously released (Fig. 2.5) but was never found in plants that received specific acaricide treatments.

Aphids exceeded their action threshold in spring in control and chemical-IPM plants only (Fig. 2.3). As a consequence a treatment of pymetrozine was applied to the latter. In this case, the areas representing percentage of aphid-infested leaf flushes above action threshold and below the population curves were highest in the control, followed by the chemical-IPM treatment and were nil for the combined-IPM strategy (Table 2.5). A similar trend was observed for *P. citrella* (Fig. 2.4) which was never detected in the combined-IPM plants. Although the two treatments of abamectin applied to the chemical-IPM plants significantly reduced the number of *P. citrella* infested leaf flushes relative to control, they did not prevent damage (Table 2.5).

Table 2.5. Estimated area under the curves of the percentages of (1) *T. urticae*-occupied rings (see Fig. 2.1), (2) symptomatic leaves occupied by *T. urticae* (see Fig. 2.2), (3) aphid-infested flushes/ring (see Fig. 2.3), and (3) *P. citrella* infested flushes/ring (see Fig. 2.4) for untreated control, combined (biological and chemical)-IPM treatment and chemical-IPM treatments.

	Untreated control	**Combined-IPM**	**Chemical-IPM**	**df; *F* ; *P***
# *T. urticae*-occupied rings	1,420.25 ± 94.8 a	180.38 ± 80.1 b	502.63 ± 202.3 b	2, 11; 22.04; <0.001
% Symptomatic leaves occupied by *T. urticae*	852.74 ± 262.6 a	36.44 ± 102.3 b	182.36 ± 51.2 b	2, 11; 6.93; 0.015
% Aphid-infested flushes/ring	485.71 ± 114.6 a	0 c	65.50 ± 23.9 b	t_6=3.894; 0.008
# *P. citrella* infested flushes/ring	207.81 ± 23.3 a	0 c	124.47 ± 5.3 b	t_6= 3.670; 0.011

Within the same row means followed by the same letter are not significantly different (Tukey for all mean separations unless *t*-test that were used for Aphid and *P. citrella* infested flushed per ring; *P* = 0.05).

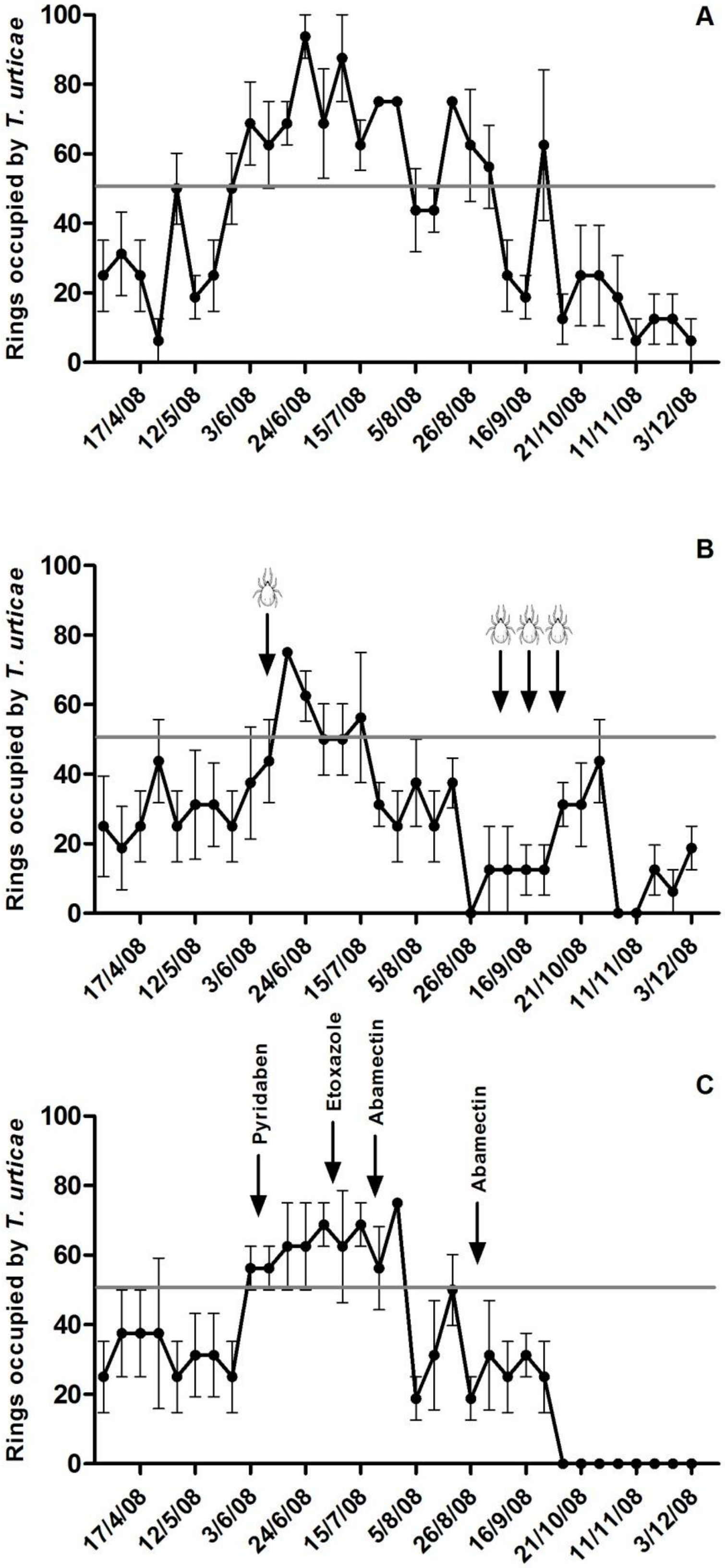


Fig. 2.1. Mean % *T. urticae*-occupied rings (±SE) in clementine plants for (A) untreated control, (B) combined (biological and chemical)-IPM treatment and (C) chemical-IPM treatment.

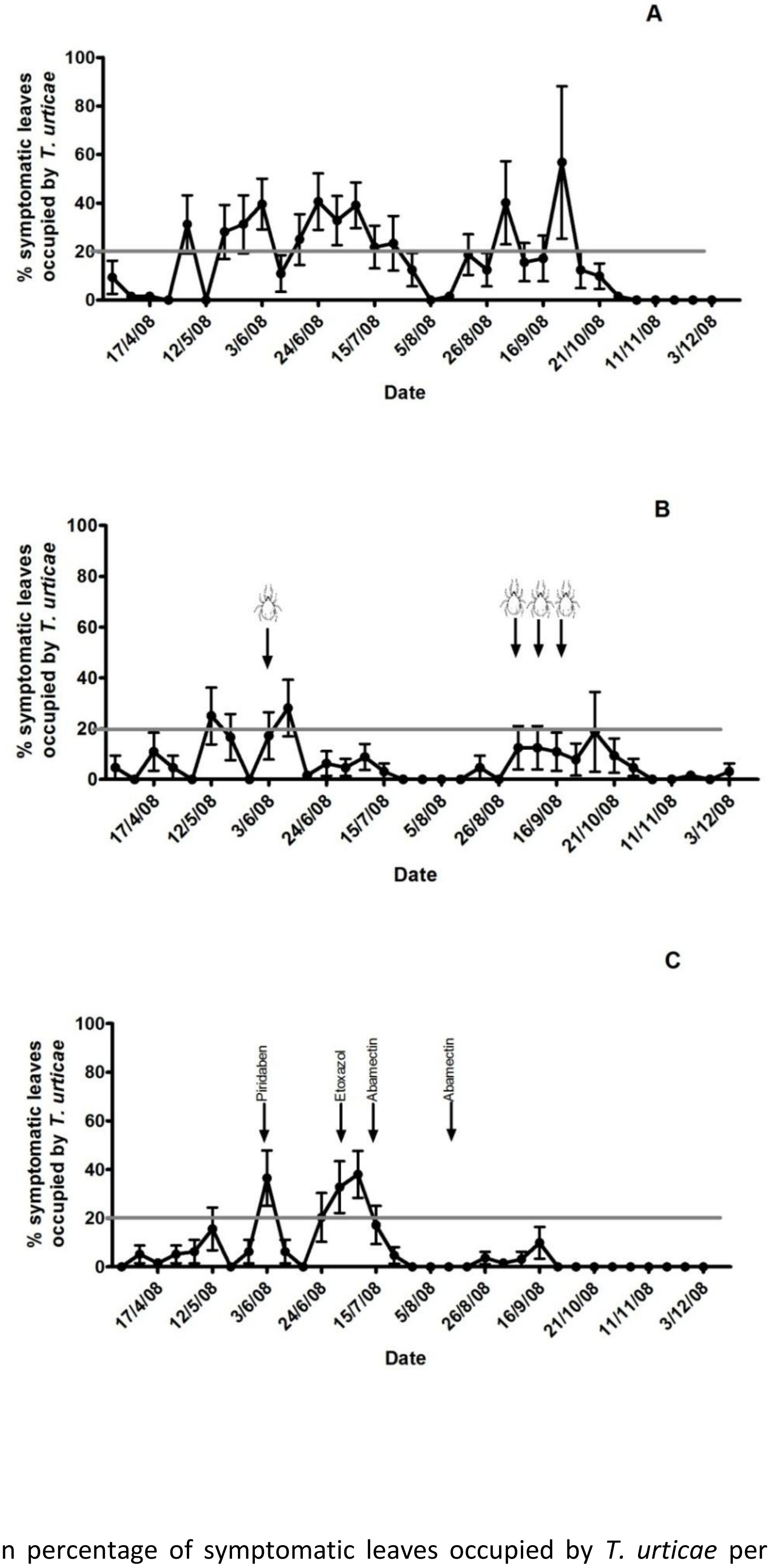


Fig. 2.2. Mean percentage of symptomatic leaves occupied by *T. urticae* per ring (±SE) in clementine plants for (A) untreated control, (B) combined (biological and chemical)-IPM treatment and (C) chemical-IPM treatment.

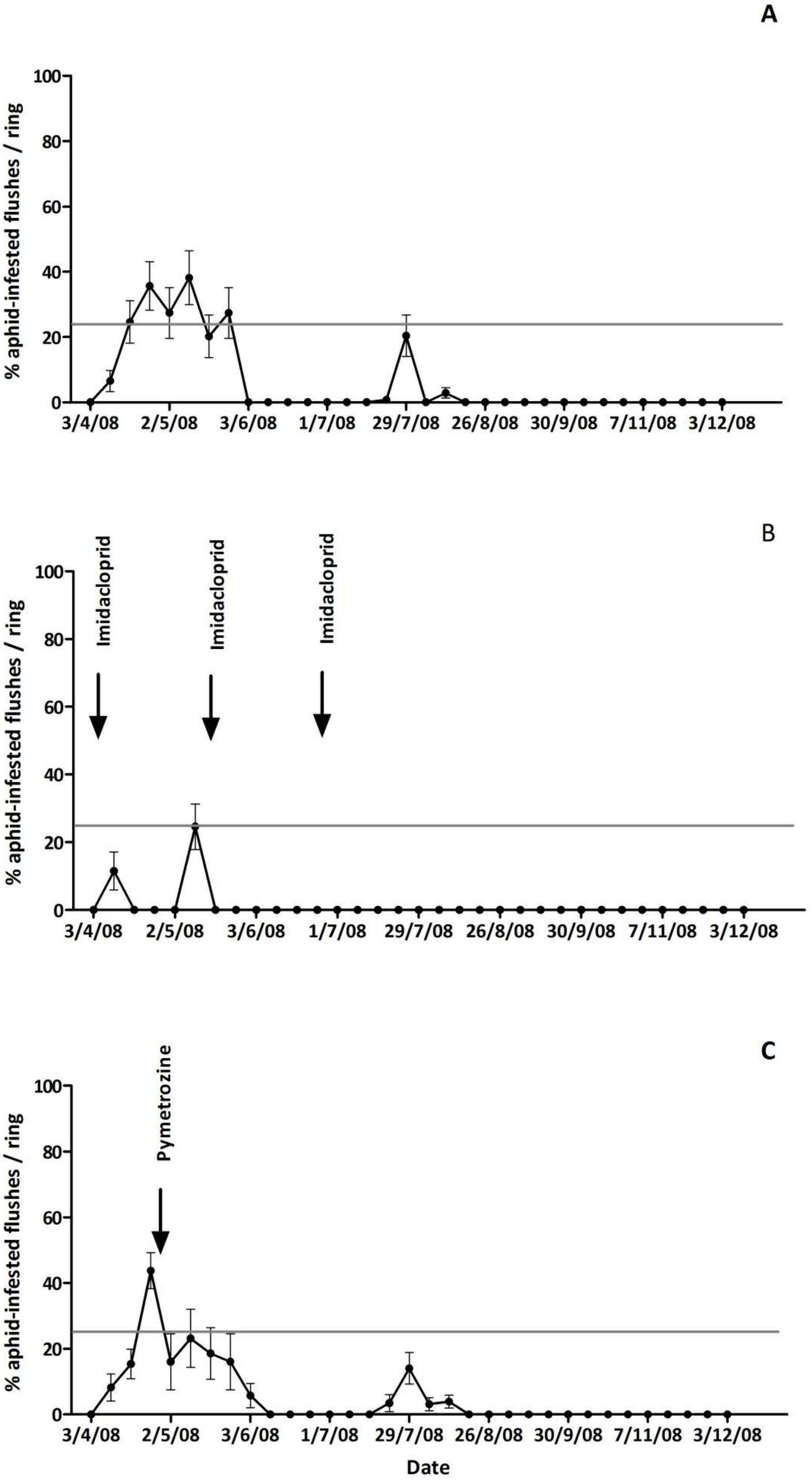


Fig. 2.3. Mean percentage of aphid-infested flushes per ring (±SE) in clementine plants for (A) untreated control, (B) combined (biological and chemical)-IPM treatment and (C) chemical-IPM treatment.

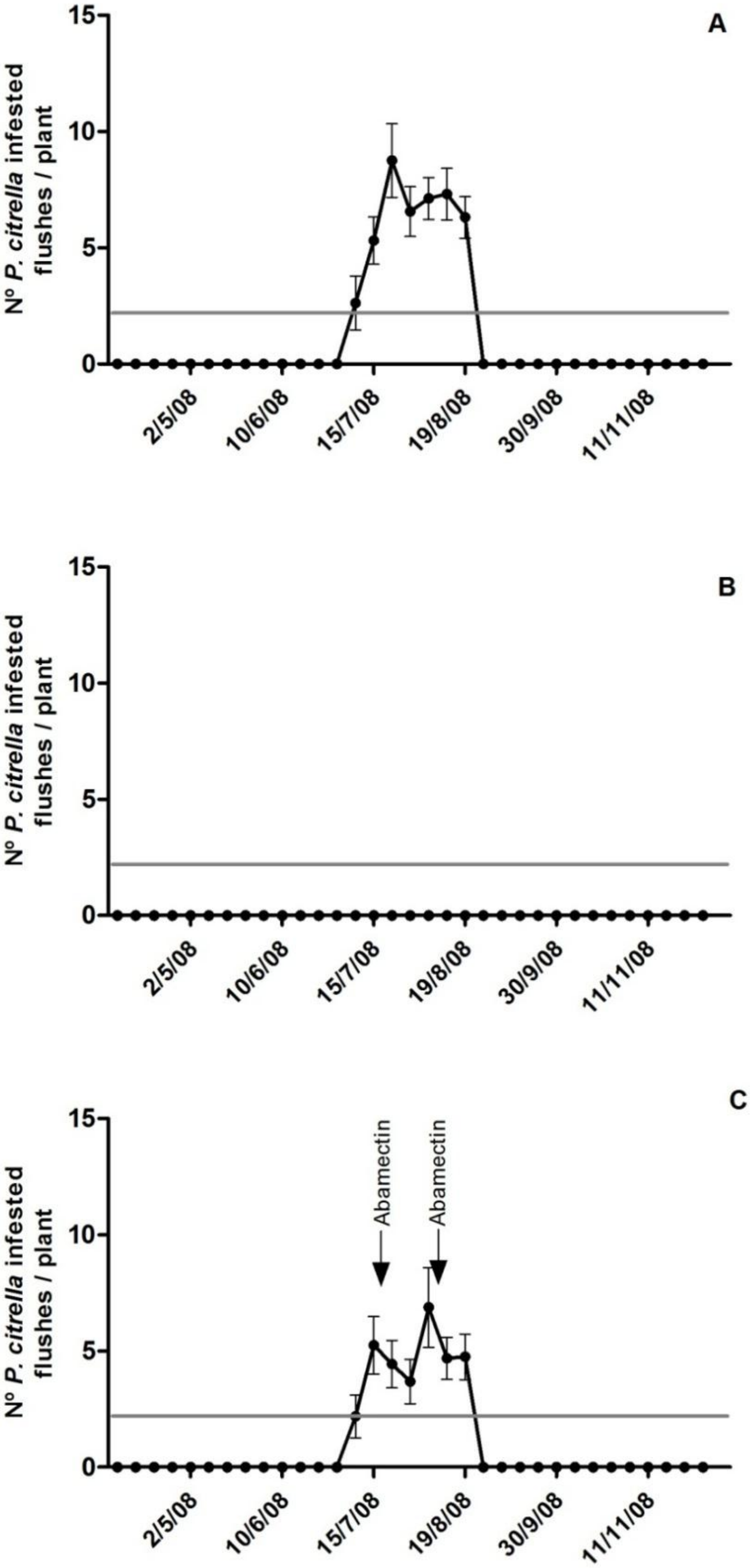


Fig. 2.4. Mean *P. citrella*-infested flushes per ring (±SE) in clementine plants for (A) untreated control, (B) combined (biological and chemical)-IPM treatment and (C) chemical-IPM treatment.

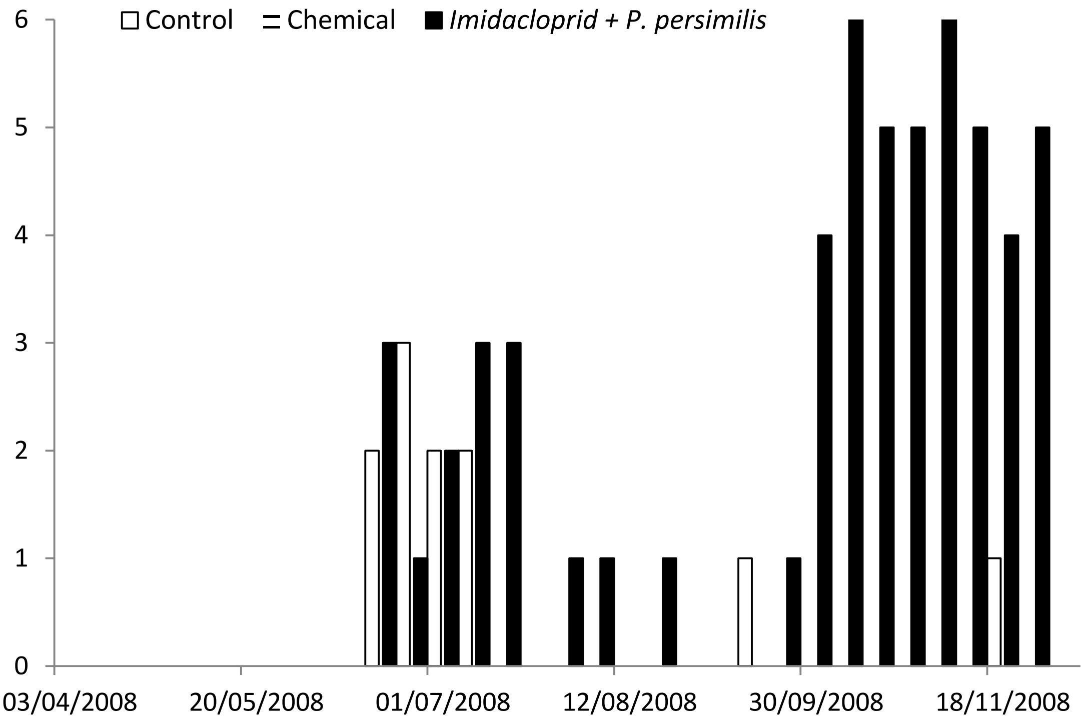


Fig. 2.5. Mean *P. persimilis*-occupied rings in clementine plants for control, combined (biological and chemical)-IPM treatment and chemical-IPM treatment.

2.5. Discussion

Results obtained in the laboratory showed that exposure of *N. californicus* and *P. persimilis* to imidacloprid through prey fed on drench-treated plants altered some demographic parameters, namely sex ratio and immature survival in *N. californicus*, and development time, peak oviposition rate, egg hatch and immature survival in *P. persimilis*. These changes resulted in lower $R_0(T)$ and r_m in *N. calfornicus* but in the case of *P. persimilis*, a higher $R_0(T)$ and an unchanged r_m were observed. Poletti et al. (2007) described an altered functional response of *N. californicus* when fed imidacloprid-treated *T. urticae* eggs. These authors described a conspicuous increase in handling time by the predator during the processes of prey identification, capture, attack, consumption, and digestion, and a decrease in the predator's attack coefficient, which indicates how fast the functional-response curve has reached its plateau. These changes led to a 55% reduction in the peak consumption of *T. urticae* eggs by *N. californicus*. This effect could explain the reduced fecundity observed by

Castagnoli et al. (2005) when *N. californicus* was exposed to imidacloprid. However, in our case, the lower $R_0(T)$ observed in *N. californicus* was not related to a lower fecundity relative to the control ($P = 0.7087$; Table 2.3) but to a dramatic decrease in immature survival (from 93.8% in control to 55.8% in imidacloprid exposed specimens). Similar results have been observed in other Phytoseiidae species including *P. macropilis* (Poletti et al. 2007). Pozzebon et al. (2011) reported that when *P. persimilis* was exposed to the neonicotinoid thiamethoxam, fecundity was reduced. However, in our case, *P. persimilis*, showed completely the opposite effects when exposed to imidacloprid. Although development time increased, peak oviposition rate was higher than in the control. This increase in fecundity was compensated by slightly lower but significantly reduced egg hatch and immature survival in treated individuals (Table 2.3). As a consequence, r_m of imidacloprid-exposed *P. persimilis* remained similar to data of the control (Table 2.4).

It is clear that data on effects of imidacloprid and other neonicotinoids on phytoseiid mites are controversial (Mizell & Sconyers 1992; James 1997; James & Price 2002; Villanueva & Walgenbach 2005; Castagnoli et al. 2005; Poletti et al. 2007; Pozzebon et al. 2011). Significant differences between closely related species emphasises the importance of not generalizing the effects of pesticides on beneficial organisms. Whether different life-styles of the two phytoseiid mites included in our study (McMurtry & Croft 1997) could explain the differences observed merits further research. Ours results suggests that a poor performance of *N. californicus* would be expected in imidacloprid-treated citrus but not for *P. persimilis*, and indeed, field results confirmed these expectations.

Laboratory results suggest that combined use of *P. persimilis* to control spider mite outbreaks and imidacloprid to control both sucking and leafminer pests could be a good alternative to purely chemical control in citrus nurseries. Field results show that both combined-IPM and chemical-IPM were equally effective against *T. urticae* (Table 2.5). However, the combined-IPM strategy better suppressed sucking pests as aphids and citrus leafminer than chemical-IPM (Table 2.5). According to previous studies (Elbert et al. 1991; Mizell & Sconyers 1992; James & Price 2002; Ako et al. 2006), imidacloprid is innocuous to *T. urticae*. Therefore, the effective control of this pest in the combined-IPM treatment should be attributed to *P. persimilis* (Fig. 2.5). This result is supported by experiments performed by Abad et al. (2010a), who reported successful control of *T. urticae* by inoculative releases of

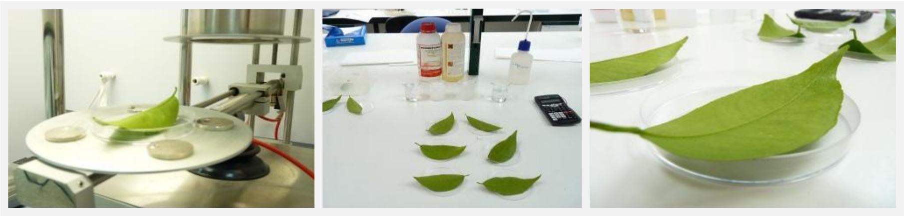
P. persimilis on young clementine plants. As expected, the chemical-IPM treatment, which included three different active ingredients effective against *T. urticae* (MAGRAMA 2012), also resulted in good control of spider mites. Interestingly, these treatments may have prevented establishment of *P. persimilis*, which, in contrast, could be recovered at the end of the season in control plants. Pyridaben is considered as moderately harmful for *P. persimilis*, whereas etoxazole and abamectin are considered harmful (Kim & Yoo 2002; Ochiai et al. 2007; Nadimi et al. 2008; Koppert).

Several studies have documented the effectiveness of imidacloprid drench applications against phloem-feeding and leaf-chewing insects (Natwick et al. 1996; Grafton-Cardwell et al. 2008; Daane et al. 2008; Chong et al. 2009; Sétamou et al. 2010; Ramzi et al. 2010; Castle & Prabhaker 2011). Our results show that drench applications of imidacloprid significantly reduced the percentage of both aphid and *P. citrella* infested flushes in clementine plants (Figs. 2.3 and 2.4; Table 2.5). These results are in agreement with those obtained by Powell et al. (2006), who reported that imidacloprid soil drenches suppressed aphid populations in Florida citrus orchards, as well as those of Ichinose et al. (2010) and Sétamou et al. (2010) who obtained good efficacies against the citrus Huanglongbing vector *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), a psyllid with feeding habits similar to those of aphids. In contrast to these satisfactory results, the application of pymetrozine against aphids in the chemical-IPM treatment reduced the percentage of aphid-infested leaf flushes compared to the control (Fig. 2.3; Table 2.5) but did not prevent damage, which is key in the nursery situation. This is in agreement with a general idea among IPM practitioners in citrus that the efficacy of available aphicides under field conditions is highly unpredictable (same authors, unpublished results). A similar situation was observed for *P. citrella*: imidacloprid-treated plants remained pest-free for the whole season (Fig. 2.4; Table 2.5). However, the two applications of abamectin, a highly effective insecticide against *P. citrella* (Urbaneja et al. 2012), in chemical-IPM plants reduced damage relative to control (Table 2.5) but did not prevent it (Fig. 2.4; Table 2.5). These results are consistent with those obtained by Sétamou et al. (2010), who demonstrated that a soil application of imidacloprid significantly reduced the infestation levels and densities of *P. citrella* starting from the second week post application.

Overall, our results show the potential of imidacloprid associated with the release of the predatory mite *P. persimilis* for the control of the main nursery pests of clementines (*T. urticae*, aphids, citrus leafminer). This neonicotinoid associated with predatory mite releases can be an interesting alternative to chemical control for successful IPM in young plants. Further work is needed to ascertain whether this pesticide could be compatible with releases of other relevant natural enemies in citrus and how this strategy could affect pest dynamics once these young plants are transferred to the orchard.

CAPÍTULO 3

COMPARATIVE TOXICITY OF DIFFERENT PESTICIDES ON THREE DIFFERENT LIFE-STYLE PHYTOSEIID MITES OCURRING IN CITRUS: *Euseius stipulatus*, *Neoseiulus californicus* AND *Phytoseiulus persimilis*



3.1. Abstract

Conservation and augmentative biological control strategies have been developed to take full advantage of the natural enemies that occur in Spanish citrus orchards. Among them, the predatory mites *Euseius stipulatus* (Athias-Henriot), *Neoseiulus californicus* and *Phytoseiulus persimilis* play an important role in the biological control of tetranychid mites. However, these predatory mites are often affected by pesticides and information about the side-effects of these products against these beneficial arthropods is essential to guarantee the efficacy of these beneficial arthropods. The side-effects of some pesticides remain unknown and the primary aim of this study is to fill this gap. We have further used this information and that collected from other sources to compare the response of these three mite species to pesticides. Based on this information, *E. stipulatus* has resulted as the most tolerant species, followed by *N. californicus* and *P. persimilis*. Therefore, using *E. stipulatus* as an indicator species in citrus may have led to the paradox of selecting presumed selective pesticides resulting in excessive impact on *N. californicus* and, especially on *P. persimilis*. Because these two latter species are considered key for the biological control of *T. urticae* in citrus in Spain, we propose to use *P. persimilis* as the right indicator of such effects in citrus instead of *E. stipulatus*. This change could have a dramatic impact on the satisfactory control of tetranychid mites in citrus in the near future.

Key words: Augmentative releases, Phytoseiidae, IPM citrus, Pesticide persistence

3.2. Introduction

As in many agricultural systems, phytoseiid predatory mites play a central role against tetranychid phytophagous mites in citrus (McMurtry 1977; McMurtry 1982). In Spain these phytophagous species are the citrus red mite *Panonychus citri* (McGregor) (Acari: Tetranychidae) and the two spotted spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) (García-Marí & Rivero 1984; Aucejo et al. 2003; Aucejo-Romero et al. 2004; Martínez-Ferrer et al. 2006; Ansaloni et al. 2008; Jacas et al. 2010). *Euseius stipulatus* (Athias-Henriot), *Neoseiulus californicus* (McGregor) and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) are their key natural enemies. On the one hand, *E. stipulatus* is the most abundant phytoseiid mite on Spanish citrus orchards (Ferragut et al. 1988; Abad-Moyano et al. 2009a). This phytoseiid is considered an omnivorous predator that can exclusively feed and successfully reproduce on pollen (Ferragut et al. 1987; Bouras & Papadoulis 2005; Pina et al. 2012) and according to McMurtry and Croft (1997) is type IV specialized pollen feeder/generalist predator life-style. The role of *E. stipulatus* as a biological control agent is limited to some tetranychid species (Ferragut et al. 1992; Grafton-Cardwell et al. 1997) is phytoseiid is considered a key player in the biological control of *P. citri* in Spain (García-Marí et al. 1983; García-Marí et al. 1986; Ferragut et al. 1988; Ferragut et al. 1992) and therefore, its conservation is a key factor for the success of IPM (Ferragut et al. 1988; Urbaneja et al. 2008). On the other hand, *N. californicus* (McGregor) and *P. persimilis* play a central role against *T. urticae* (van Lenteren & Woets 1988; Grafton-Cardwell et al. 1997; van Lenteren 2000; Abad-Moyano et al. 2010a). According to McMurtry and Croft (1997), *N. californicus* is a type II specialized predator of tetranychid mites whereas *P. persimilis* is type I specialized predator of *Tetranychus* species. These two predatory mite species are consistently encountered among colonies of *T. urticae* in citrus (McMurtry 1977; Abad-Moyano et al. 2009a). Conservation and augmentative biological control strategies have been developed to take advantage of these species in citrus, both in nurseries and in the field (Wilson 1966; Landis et al. 2000; Jacas & Urbaneja 2010). In the former situation, seasonal inoculative releases of either *P. persimilis* or *N. californicus* in combination with selective pesticides have resulted highly effective (Abad-Moyano et al. 2010a; Argolo et al. 2012). In the case of orchards, and depending on the citrus species considered, conservation biological control strategies combining the management of the ground cover with other selective pest management strategies have also yielded good results (Aguilar-Fenollosa et al. 2011a; Aguilar-Fenollosa et al. 2011b; Aguilar-Fenollosa et al. 2011c). Although the range of available selective pest control methods has dramatically increased in recent years in citrus (Jacas & Urbaneja 2010; Vacas et al. 2012; Vanaclocha et al. 2012; Juan-Blasco et al. 2012) chemical control is still needed in some cases (Urbaneja et al. 2012). To identify the most selective biocides that could be used in combination with biological control strategies, it is very important to know the side effects of these products on the most relevant natural enemies for each specific crop (Hassan 1977; Hassan et al. 1985; Hassan & Oomen 1985; Hassan et al. 1988; Sterk et al. 1999; Blümel et al. 2002). The effect of many pesticides used in citrus on the three phytoseiid species mentioned above is already available (Bellows & Morse 1988; Bellows et al. 1992; Costa-Comelles et al. 1994; Jacas & Garcia-Marí 2001; Pascual-Ruiz 2005; San Andrés et al. 2006; Urbaneja et al. 2008; Urbaneja et al. 2012; Koppert 2012; Biobest 2012). However, the effect of few products remains unknown. The first objective of this study has been to fill this gap. Further, with the complete dataset we have compared the relative toxicity of pesticides on these three different life-style phytoseiids. This information should eventually help us to identify whether any of these species could be used as indicator of pesticide side-effects on predatory mites occurring in citrus.

3.3. Material and methods

3.3.1. Mite cultures

Tetranychus urticae colonies were initiated with individuals collected in clementine mandarin orchards in the region of La Plana (Castellon de la Plana, Spain) and were maintained on lemon fruits in a climatic chamber 25 ± 2 °C, 60 ± 10% RH and a 16:8 h L:D photoperiod.

Individuals of *E. stipulatus* were collected from clementine mandarin orchards infested with *T. urticae* and *P. citri* in Moncada. *Neoseiulus californicus* and *Phytoseiulus persimilis* were originally purchased from Koppert Biological Systems (Spical® and Spidex®, respectively). *Euseius stipulatus* colony was maintained on bean leaves *Phaseoulus vulgaris* (L.) (Fabaceae) with *Carpobrotus edulis* (L.) (Aizoaceae) pollen. Strips of wet tissue were placed on bean leaves along their periphery to ensure a constant water supply to the phytoseiids and to prevent escape and contamination with other mite species. The rearing units of *P. persimilis* and *N. californicus* were similar to those described by Overmeer (1985). They consisted of a piece of hard black plastic placed onto a water saturated sponge. The sponge with the plastic piece was introduced in a plastic tray with water. The plastic borders were covered with thin sheets of tissue paper to ensure a constant water supply to the phytoseiids, to fix the plastic piece to the sponge, and to prevent phytoseiids from escaping. Twice a week, *T. urticae* infested clementine leaves were added to each phytoseiid rearing unit. To avoid rearing contamination, each species was kept in different climatic chambers [25 ± 1°C, 80 ± 5% RH and 16:8 h (L:D) photoperiod].

3.3.2. Pesticides

The pesticides included in this assay were selected from those authorized in citrus by the Spanish Ministry of Agriculture (MAGRAMA 2012). Moreover, we included one pesticide of interest for future IPM strategies in citrus, spirotetramat. This pesticide is being used to control *A. aurantii* in different citrus areas of the world (Grafton-Cardwell et al. 2007), while in Spain it is recently registered (MAGRAMA 2012). The concentration of spirotetramat used in our assays was the maximum label registration proposed by Bayer Crop Science (Valencia, Spain). Maximum authorized concentrations in citrus as indicated on the label were used in our assays (Table 3.1).

Table 3.1. Pesticide formulations evaluated in the present study

Active ingredient	**Trade name**	**Manufacturer**	**Max. authorized dose in citrus***	**Target pests**
Abamectin (18 gL^{-1} EC)	Cable©	Ender Iberica S.A.	40mL hL^{-1}	Mites, Lepidopters
Spirotetramat (150 gL^{-1} OD)	Movento©150OD	Bayer Cropscience, S.L.	50mL hL^{-1}	Aphids, White flies, Scales
Etofenprox (300 gL^{-1} EC)	Trebon 30 LE©	Certis Europe BV, Spain	100mL hL^{-1}	Fruitflies, Aphids
Etoxazole (110 gL^{-1} SC)	Borneo©	Kenogard, S.A.	50mL hL^{-1}	Mites
Mineral Oil (850 gL^{-1} EC)	Sunspray Ultrafine©	Agrichem, S.A.	1.5 L hL^{-1}	White flies, Mealybugs

EC emulsifiable concentrate, SC suspension concentrate, OD oil dispersion
*Spanish Ministry of Agriculture (MAGRAMA 2012).

3.3.3. Experimental unit (arena)

Clementine (*Citrus clementina* Hort. ex Tan. var. Nules) leaves fully-expanded (approximately 5 cm length) were obtained from unsprayed potted trees grown under controlled environmental conditions (25 ± 2°C, 65 ± 10% RH) in an insecticide-free greenhouse located at the Valencian Institute of Agricultural Research (IVIA).

The experimental arenas consisted of a treated leaf placed upside down on a water-saturated sponge previously lined with a paper towel, which was placed on a plastic container with water (14.0 × 14.0 × 7.5 cm). Strips of wet cotton were placed on the clementine leaf along its periphery to ensure a constant water supply to the phytoseiids and to prevent mites escape. Adults were transferred into each experimental unit directly from the colony with a fine brush. During all the experiments nymphs and adults of *T. urticae* were introduced in each experimental arena, as food for *P. persimilis* and *N. californicus*. Pollen of *C. edulis* was provided as a food source for *E. stipulatus*.

Because we were interested in estimating the duration of the harmful effect before release, we decided to use this stage instead of immature stages, as recommended by the International Organization for Biological Control (IOBC) Working Group 'Pesticides and Beneficial Organisms' standards (Hassan & Oomen 1985; Sterk et al. 1999).

3.3.4. Assays and evaluation

Three different bioassays were carried out. Each bioassay consisted of eight replicates per treatment. Ten adults were used per replicate. Pesticides were applied to fresh citrus leaves using a Potter Spray Tower (Burkard Scientific Ltd, Uxbridge, UK). In all bioassays, 2 ml of the product dilution was sprayed at 200 kPa, resulting in a deposit of 2 mg cm^{-2}. A distilled water spray control was included. Adults were exposed to different residues of the selected pesticides. Fresh, 7-, 14- and 21-day-old residues were assayed.

Predatory mites were considered as dead at the end of this period if no moves were observed under a binocular. When no significant differences were found between a pesticide and the control, the pesticide was excluded from the following residual experiment.

For the fresh residue assays, as soon as treated leaves were dried, the predatory mites were transferred to each experimental unit as described above. For the 7-, 14-, 21-day-old residues, the petiole of the treated leaves was inserted in a floral foam containing water to keep the leaf turgid until use. During this storage period, leaves were kept in a climatic chamber at 20 ± 1°C, 60 ± 10% RH and a photoperiod of 16:8 h (L:D). The experimental units containing the predatory mites were incubated in a climatic chamber until the end of the assay at 25 ± 1°C, 70 ± 10% RH and a photoperiod of 16:8 h (L:D).

3.3.5. Comparison of relative toxicity of pesticides

A list of the side effects of the pesticides recommended in IPM in citrus on *E. stipulatus*, *P. persimilis* and *N. californicus* was established based on the information compiled from both the literature and the internet webpages of companies involved in the production of these predatory mites. These values were always referred to IOBC categories (Hassan 1994; Sterk et al. 1999).

3.3.6. Data analysis

For each experiment, percentage mortality for *E. stipulatus*, *N. californicus* and *P. persimilis* in treated arenas was compared with the control by means of ANOVA, and the LSD test was used for mean separation at $P < 0.05$. When needed, data were angular transformed to achieve homoscedasticity and normal distribution of residues. When significant differences were found ($P < 0.05$), between control and pesticide treatments, mortality was corrected using Abbott's formula. Subsequently, data were interpreted according to the IOBC standards (Sterk et al. 1999; Hassan 1994), which include four categories: 1) harmless, mortality lower than 30%; 2) slightly harmful, between 30 - 79%; 3) moderately harmful, between 80 - 99%; and 4) harmful, mortality higher than 99%. SPSS 19.0 software was used for statistical analysis.

To compare the relative toxicity of pesticides (expressed as IOBC categories) among the three phytoseiids included in this study, toxicity expressed as IOBC values for pesticide and predatory mite were compared using correlation analysis.

3.4. Results

3.4.1. Fresh residue

Significant differences were found between control and treatment groups for all species tested (Tables 3.2 to 3.4). Abamectin, etofenprox and spirotetramat produced similar mortality values on the three species. In the case of abamectin and etofenprox mortality values were always above 80% mortality, whereas spirotetramat caused around 30% mortality. Etoxazole behaved similarly for *E. stipulatus* and *N. californicus* and resulted in around 50% mortality, whereas it caused 34% mortality on *P. persimilis*. However, this species was much more sensitive to mineral oil (64% mortality) than *N. californicus* (36%) and *E. stipulatus* (19%).

3.4.2. Aged- residues

Only abamectin and etofenprox behaved differently to the control for 7-day-old residues for the predatory mite species tested (Tables 3.2 to 3.4). However, abamectin was much less toxic than etofenprox and lost its toxicity against the three species considered one week later (14-day-old residue). Etofenprox resulted quite toxic especially for *E. stipulatus* and *N. californicus*, (99% and 95% mortality, respectively for 7-day-old residue) and did not lose its toxicity even 21 days after application (mortality above 79% in all cases).

3.4.3. Comparison of relative toxicity of pesticides

To establish whether the three species included in this study responded similarly to pesticides, correlation analysis was applied to IOBC values for each pair (Table 3.5). All correlations were significant (*E. stipulatus* vs. *N. californicus*: $F = 6.71$; $df = 1, 21$; $P = 0.018$, *E. stipulatus* vs. *P. persimilis*: $F = 7.31$; $df = 1, 21$; $P = 0.014$, and *N. californicus* vs. *P. persimilis*: $F = 21.01$; $df = 1, 21$; $P < 0.001$). However, correlation coefficients were lower for comparisons including *E. stipulatus* ($r^2 = 0.251$ when compared to *N. californicus*, and $r^2 = 0.268$ when compared to *P. persimilis*) than when comparing *N. californicus* and *P. persimilis* ($r^2 = 0.501$) (Figure 3.1).

Table 3.2. Means (± SEM) of mortality percentages of *E. stipulatus* adults after exposure to different residues and untreated control.

Product	**Fresh residue**	**7-day-old**	**14-day-old**	**21-day-old**
Abamectin	92.5 ± 4.1 a (92.1)	33.8 ± 3.5 b (31.2)	6.3±1.8 b (-)	
	[3]	[2]	[1]	
Spirotetramat	37.5 ± 5.9 c (34.2)	10.0 ± 2.7 c (-)		
	[2]	[1]		
Etofenprox	97.5 ± 1.6 a (97.4)	98.8 ± 1.3 a (98.7)	81.3±5.2 a (80.8)	78.8±5.5 a (78.5)
	[3]	[3]	[3]	[2]
Etoxazole	56.3 ± 5.6 b (53.9)	12.5 ± 1.6 c (-)		
	[2]	[1]		
Mineral Oil	18.8 ± 4.0 d (14.5)	12.5 ± 1.6 c (-)		
	[1]	[1]		
Control	3.8 ± 1.8 e	3.8±1.8 c	2.5±1.6 b	1.3±1.3 b
F (*df*)	83.7 (5, 47)	276.5 (5, 47)	33.7 (2, 23)	t_{14}= 20.1
P	<0.001	<0.001	<0.001	<0.001*

Corrected mortality (Abbott) between parentheses is showed. IOBC toxicity category between brackets is showed: [1] harmless, mortality lower than 30%; [2] slightly harmful, mortality between 30 and 79%; [3] moderately harmful, mortality between 80 and 99%; [4] harmful, mortality higher than 99%. Means within columns, data followed by the same letters are not significantly different ($P < 0.05$; LSD test,*Student's t-test)

Table 3.3. Means (± SEM) of mortality percentages of *N. californicus* adults after exposure to different residues and untreated control.

Product	**Fresh residue**	**7-day-old**	**14-day-old**	**21-day-old**
Abamectin	87.5 ± 5.6 a (87.3)	26.3 ± 2.6 b (24.4)	5.0 ± 2.7 b (-)	
	[3]	[1]	[1]	
Spirotetramat	27.5 ± 2.5 c (26.6)	15.0 ± 3.8 bc (-)		
	[1]	[1]		
Etofenprox	100.0 ± 0.0 a (100)	95.0 ± 5.0 a (94.9)	100 ± 0.0 a (100)	97.5±1.6 a (97.5)
	[4]	[3]	[4]	[3]
Etoxazole	47.5 ± 4.1 b (46.8)	10.0 ± 3.3 c (-)		
	[2]	[1]		
Mineral Oil	36.3 ± 6.5 bc (35.4)	11.3 ± 4.0 c (-)		
	[2]	[1]		
Control	1.3 ± 1.3 d	2.5 ± 1.6 c	0 b	0 b
F (*df*)	98.7 (5, 47)	93.9 (5, 47)	180.1 (2, 23)	
P	<0.001	<0.001)	<0.001)	<0.001

Corrected mortality (Abbott) between parentheses is showed. IOBC toxicity category between brackets is showed: [1] harmless, mortality lower than 30%; [2] slightly harmful, mortality between 30 and 79%; [3] moderately harmful, mortality between 80 and 99%; [4] harmful, mortality higher than 99%. Means within columns, data followed by the same letters are not significantly different ($P< 0.05$; LSD test,*Student's t-test)

Table 3.4. Means (± SEM) of mortality percentages of *P. persimilis* adults after exposure to different residues and untreated control.

Product	**Fresh residue**	**7-day-old**	**14-day-old**	**21-day-old**
Abamectin	96.3 ± 2.6 a (96.2)	41.3 ± 4.0 b (40.5)	11.3 ± 3.0 b (-)	
	[3]	[2]	[1]	
Spirotetramat	25.0 ± 2.7 e (23.1)	3.8 ± 2.6 c (-)		
	[1]	[1]		
Etofenprox	81.3 ± 2.0 b (80.8)	76.3 ± 2.6 a (75.9)	80.0±2.7 a (79.5)	82.5 ± 2.5 a (81.8)
	[3]	[2]	[2]	[3]
Etoxazole	33.8 ± 1.8 d (32.1)	3.8 ± 2.6 c (-)		
	[2]	[1]		
Mineral Oil	63.8 ± 4.2 c (62.8)	12.5 ± 2.5 c (-)		
	[2]	[1]		
Control	2.5 ± 1.6 f	1.3 ± 1.3c	2.5 ± 1.6 b	3.8 ± 2.6 b
F (*df*)	180.6 (5, 47)	112.3 (5, 47)	323.1 (2, 23)	t_{14}= 21.7
P	<0.001	<0.001	<0.001	<0.001*

Corrected mortality (Abbott) between parentheses is showed. IOBC toxicity category between brackets is showed: [1] harmless, mortality lower than 30 %; [2] slightly harmful, mortality between 30 and 79 %; [3] moderately harmful, mortality between 80 and 99 %; [4] harmful, mortality higher than 99 %. Means within columns, data followed by the same letters are not significantly different (P< 0.05; LSD test,*Student's t-test)

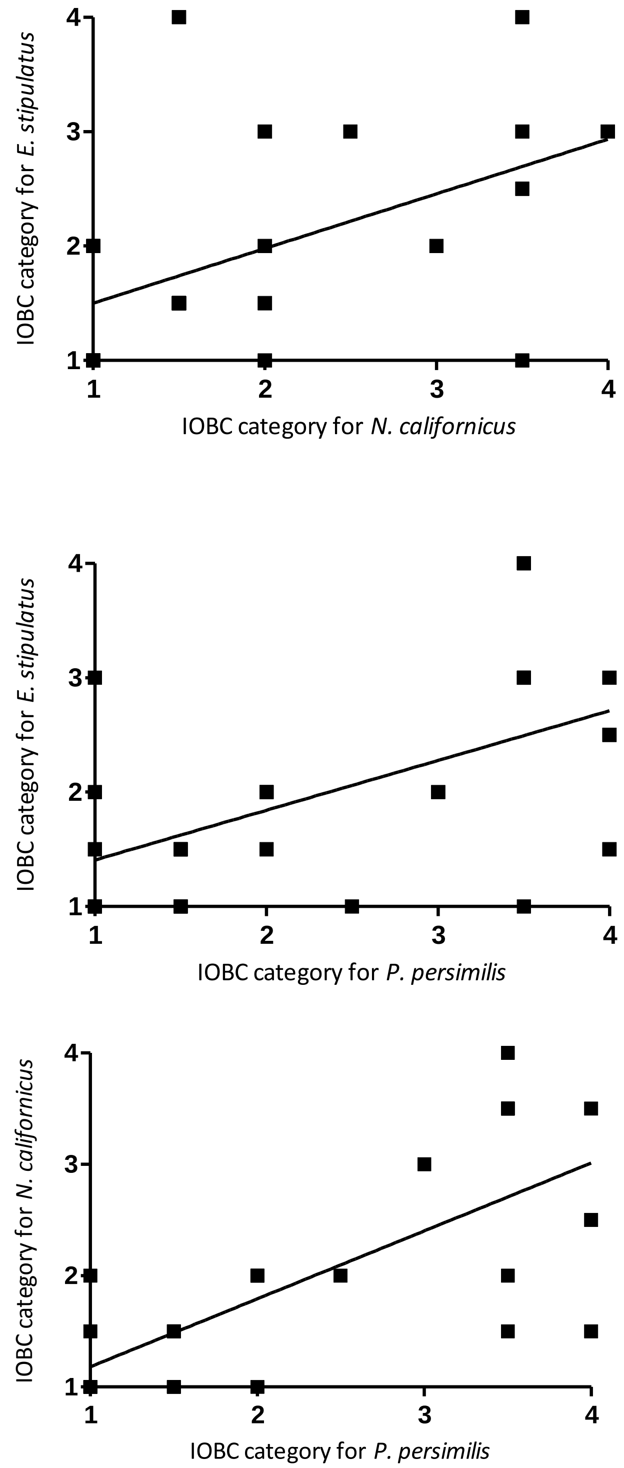


Fig. 3.1. Relationships of toxicities for the predatory mites *E. stipulatus*, *N. californicus* and *P. persimilis* based on the IOBC categories as shown in Table 3.5.

3.5. Discussion

The results included in this study have allowed us to complete the list of side-effects of those pesticides authorized in citrus under IPM label in Spain (MAGRAMA 2012). This information will allow the selection of the most appropriate pesticide against citrus key pests taking into account its side-effects on predatory mites. Moreover, our results on the persistence of the toxic effects of pesticides will allow a better timing of the releases of natural enemies sensitive to fresh residues but not to older residues of the same pesticide. This type of information is becoming increasingly important in citrus because augmentative releases of mass-reared beneficial arthropods are being promoted in citrus (Argolo et al. 2012). For example, etofenprox and abamectin resulted highly toxic for the three mite species considered (Table 3.5). However, etofenprox showed a persistence of more than 3 weeks compared to 1-2 weeks, depending on the species, for abamectin (Table 3.5).

Although the three species included in this study responded similarly to most of the pesticides tested, some important differences were found (Table 3.5). Generally, *E. stipulatus* was more tolerant to pesticides than the other two phytoseiids and this may be related to their different life-style. 31% of the pesticides included in Table 5 were less toxic for *E. stipulatus* than for *P. persimilis* and *N. californicus*. The latter species followed in the ranking and *N. californicus* resulted less affected by pesticides than *P. persimilis* in 39% of the cases. Nevertheless, to date *E. stipulatus* has been used as indicator species of pesticide side-effects for predatory mites in citrus in Spain (Jacas & Urbaneja 2010). This fact may have had dramatic effects on the biological control of *T. urticae*.

In general, the key to effective biological control may be tactics that enhance the relative abundance of the most effective predators within the predator community (Straub & Snyder 2006). In our case, these predators are type I and II entomophagous phytoseiids as *P. persimilis* and *N. californicus*. Recent studies have shown that these two species, even if quantitatively less represented in the predatory mite guild occurring in citrus than *E. stipulatus* (Abad-Moyano et al. 2009a; Aguilar-Fenollosa et al. 2011b), are key in the regulation of the populations of *T. urticae* (Aguilar-Fenollosa et al. 2011a). Furthermore, new studies (same authors, unpublished results) have shown that *P. persimilis* consistently appears in citrus trees where pesticide treatment regime is low, such as those where *T.*

urticae management is based on the use of *Festuca arundinacea* Schreber (Poaceae) ground cover (Aguilar-Fenollosa et al. 2011c). Therefore, using *E. stipulatus* as an indicator species in citrus may have led to the paradox that the use of presumed selective pesticides has resulted in the disappearance of *P. persimilis* and *N. californicus* from the trees and, as a consequence, in a deficient control of *T. urticae* in clementines, as it is the case too often in the Region of Valencia (same authors, unpublished results). For this reason, we propose *E. stipulatus* not to be further considered as the indicator species for pesticide side-effects in citrus and focus on *P. persimilis*, which is the most sensitive and relevant phytoseiid species occurring in citrus orchards in Spain. Such a change can have a dramatic impact on the survival of *P. persimilis* and *N. californicus* in our orchards and therefore in the satisfactory control of tetranychid mites in citrus in the near future.

Table 3.5. List of side effects of the pesticides recommended in integrated pest management in citrus on the predatory mites *E. stipulatus, N. californicus* and *P. persimilis*.

	E. stipulatus		***N. californicus***		***P. persimilis***	
Product	IOBC category	Persistence (days)	IOBC category	Persistence (days)	IOBC category	Persistence (days)
Abamectin	3 (3, 10)	14	3 - 4 (1, 2)	7	3 - 4 (1, 2)	14
Mineral oil	1 (8, 10)	-	2 (1, 2, 4)	7	2 - 3 (1, 2)	7
Acetamiprid	1 (7)	-	3 - 4 (1, 2)	5	3 - 4 (1, 2)	7
Azadirachtin	1 - 2 (3)	-	1 - 2 (1, 2)	-	1 - 2 (1, 2)	0
B. thuringiensis	1 (3)	-	1 (1, 2)	-	1 (1, 2)	0
Clofentezine	1 - 2 (3)	-	1 - 2 (1, 2, 4)	0	1 (1, 2)	0
Chlorpyrifos	2 (3, 5, 6)	-	3 (1, 2)	2	3 (1, 2)	0
Etofenprox	3	+21	4	+21	3 - 4 (1)	+21
Etoxazole	2	7	2	7	2	7
Fenpyroximate	3 (7)	-	2 - 3 (1, 2)	5	4 (1, 2)	14
Hexythiazox	1 (3)	-	1 (1, 2)	0	1 - 2 (1, 2)	0
Imidacloprid	2-3 (3)	-	3 - 4 (1, 2)	5	4 (1, 2)	14
Chlorpyrifos-methyl	3 (3)	-	2 (1)	2	1 (1)	0
Pymetrozine	1(7)	-	1 (1, 2)	0	1 - 2 (1, 2)	0
Pyridaben	4 (3)	-	3 - 4 (1, 2)	5	3 - 4 (1, 2)	14
Pirimicarb	1 - 2 (3)	-	1 - 2 (1, 2)	0	1 - 2 (1, 2)	3
Pyriproxifen	1 (3)	-	1 (1, 2)	0	1 (1, 2)	0
Propargite	4 (3)	-	1 - 2 (1, 2)	0	3 - 4 (1, 2)	3
Spinosad	2 (9)	-	1 (1, 2)	0	2 (1, 2)	7
Spirodiclofen	1 – 2 (7)	-	2 (2)	-	2 (2)	14
Spirotetramat	2	7	1	-	1	-
Tebufenpyrad	1 - 2 (8)	-	1 - 2 (1, 2, 4)	0	4 (1, 2)	2
Thiametoxam	-	-	2 (1, 2)	-	3 - 4 (1, 2)	+14

IOBC categories: *1* harmless, mortality lower than 30 %; *2* slightly harmful, mortality between 30 and 79 %; *3* moderately harmful, mortality between 80 and 99 %; *4* harmful, mortality higher than 99 %.

References: (1) (Koppert 2012); (2) (Biobest 2012); (3) (Jacas & Garcia-Marí 2001); (4) (Urbaneja et al. 2008); (5) (Bellows et al. 1992); (6) (Bellows & Morse 1988); (7) (Urbaneja et al. 2012); (8) (Pascual-Ruiz 2005); (9) (San Andrés et al. 2006) (10) (Costa-Comelles et al. 1994)

CAPÍTULO 4

EFFECT OF POLLEN QUALITY ON THE EFFICACY OF TWO DIFFERENT LIFE-STYLE PREDATORY MITES AGAINST *Tetranychus urticae* IN CITRUS

Una versión modificada de este capítulo ha sido publicada en Biological
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4.1. Abstract

Cover crops can serve as a reservoir of natural enemies by supplying alternative food sources as pollen. In turn, pollen quality and availability can modulate phytoseiid communities. In clementine trees associated with a cover crop of *Festuca arundinacea* Schreber, these communities were more diverse than those associated with a multifloral wild cover crop. As a consequence, the former had a better regulation of *Tetranychus urticae* populations than the latter. Longer provision of higher quality pollen in the multifloral cover relative to *F. arundinacea* is suspected to interfere with the biological control of *T. urticae* by specific phytoseiid predators (*Phytoseiulus persimilis* and *Neoseiulus californicus*) by enhancing the less efficient generalist pollen feeder *Euseius stipulatus* which is a superior intraguild predator. To determine whether pollen quality is behind these results, the effect of the provision of two different pollens (*Carpobrotus edulis* (L.) L. Bolus and *F. arundinacea*) on the efficacy of two phytoseiid species (*E. stipulatus* and *N. californicus*) to regulate *T. urticae* populations has been studied under semi-field conditions. Results suggest that pollen provision does not enhance the ability of these phytoseiids to reduce *T. urticae* populations. However, *C. edulis* pollen resulted in explosive increases of *E. stipulatus* numbers that did not occur with *F. arundinacea* pollen. Therefore, poor quality pollen may prevent pollen feeders from reaching high numbers in the field. This effect could benefit phytoseiid species suffering intraguild predation by *E. stipulatus* explain field results.

Key words: Cover crop, Conservation Biological Control, Phytoseiidae, Omnivory, Generalist predator, Specialist predator

4.2. Introduction

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important pest of citrus in Spain (Jacas et al., 2010). Mite populations in clementine mandarins (*Citrus clementina* Hort. ex Tan.) can increase rapidly and cause severe economic losses mainly due to fruit scarring (Ansaloni et al. 2008). Until now, *T. urticae* control in Spain has been mainly based on pesticides and the extensive use of these acaricides has led to many management problems (Martínez-Ferrer et al. 2006; Urbaneja et al. 2008). Conservation biological control strategies through habitat management have been proposed as an efficient and sustainable alternative to pesticide use in different crops and regions (Landis et al. 2000; Jonsson et al. 2008). Indeed, habitat management of agricultural systems using non-crop plant species as ground covers have been reported as an effective option to control mite populations in citrus (Jacas & Urbaneja 2010; Aguilar-Fenollosa et al. 2011a; Aguilar-Fenollosa et al. 2011b; Aguilar-Fenollosa et al. 2011c).

Ground covers can affect arthropod population dynamics and serve as reservoirs of natural enemies through provision of food sources when prey food is rare or absent, by supplying a shelter for overwintering or aestivation or a refuge from agricultural practices, and even for breeding (Alston 1994; Liang & Huang 1994; Jonsson et al. 2008; Mailloux et al. 2010). However, cover crops can harbor both beneficial and harmful arthropods and if they are not properly managed, harmful arthropods can develop as a pest (Aucejo et al. 2003). Different studies on cover crops have proposed the use of some perennial grasses (Poaceae) because they are unsuitable hosts for phytophagous mites (Flexner et al. 1991; Alston 1994; Barbosa 1998; Nyrop et al. 1998) and specifically for *T. urticae* in clementine mandarin orchards (Aucejo et al. 2003). Recently, a mono-specific cover of the perennial grass *Festuca arundinacea* Schreber (Poaceae) has demonstrated to improve the regulation of *T. urticae* in clementine trees when compared with a wild cover or bare soil, both by reducing the successful settlement of *T. urticae* in clementine mandarin trees (Aguilar-Fenollosa et al. 2011a) and by enhancing the populations of more efficient specialist predators relative to generalist pollen feeder predators (Aguilar-Fenollosa et al. 2011b). Furthermore, the use of *F. arundinacea* as a cover crop is economically more cost effective than wild cover or bare soil in clementine mandarins (Aguilar-Fenollosa et al. 2011c) and it is being currently promoted in Spanish citrus orchards (Jacas & Urbaneja 2010). *Neoseiulus californicus*

(McGregor) and *Euseius stipulatus* (Athias-Henriot) are two phytoseiid mites frequently found both in the canopy and in the *F. arundinacea* cover crop in mandarin clementine groves (Abad-Moyano et al. 2009a; Aguilar-Fenollosa et al. 2011b). The tetranychid specialist *N. californicus* is commercially available as a biological control agent against tetranychid mites (McMurtry & Croft 1997), and when feeding exclusively on pollen, this predator can survive and even reproduce (Castagnoli & Simoni 1999; Sazo et al. 2006; Ragusa et al. 2009). *Euseius stipulatus* is an omnivorous predator that can exclusively feed and successfully reproduce on pollen (Ferragut et al. 1987; Bouras & Papadoulis 2005) and its role as a biological control agent is limited to some tetranychid species, other than *T. urticae*, as *Panonychus citri* (McGregor) (Acari: Tetranychidae) (Ferragut et al. 1992; Grafton-Cardwell et al. 1997; González-Fernández et al. 2009).

The relationship between pollen availability and satisfactory pest control has been studied in different systems. In San Joaquin Valley citrus groves (California, US), increases of *Euseius* (=*Amblyseius*) *hibisci* (Chant) populations were associated with peaks of wind-borne pollen, which allowed this species to satisfactorily control the citrus red mite *P. citri* (Kennett et al. 1979). Citrus growers in China used pollen of the Asteraceae *Agerantum conyzoides* L., as an alternative food mainly for *Amblyseius* spp., to benefit *P. citri* control (Liang & Huang 1994). *Euseius stipulatus* and *N. californicus* increased their numbers and reduced the populations of the herbivore *Oligonychus perseae* Tuttle, Baker and Abbatiello in avocado groves supplied with corn pollen in Spain (González-Fernández et al. 2009). However, ample pollen supply in Spanish clementine mandarin orchards is suspected to enhance *E. stipulatus* populations to an extent that could impair the control exerted on *T. urticae* by other phytoseiids specialized in tetranychid species (Aguilar-Fenollosa et al. 2011a). In a semi-field assay, Abad-Moyano et al. (2010b; 2010c) found negative effects of *E. stipulatus* presence on the efficacy in spider mite suppression by *N. californicus* and on population development of *Phytoseiulus persimilis* Athias-Henriot. These experiments suggested that lethal and non-lethal intraguild interactions between *E. stipulatus* and *P. persimilis* and *N. californicus*, respectively, could contribute to the deficient natural control of *T. urticae* often observed in Spanish commercial clementine orchards (usually grown on bare soil or in association with a wild cover).

Furthermore, Aguilar-Fenollosa et al. (2011a) described a higher abundance of *E. stipulatus* in clementine trees when a multifloral cover was used compared with a *F. arundinacea* cover crop. These authors suggested that the extended pollination period in multifloral cover conditions could have promoted an increase in numbers of this superior intraguild predator when compared with *F. arundinacea* that blooms in spring only. However, increases in numbers of pollen feeder predators could be attributed not only to an extended blooming period but also to the nutritional quality of the pollen, its grain structure, or even the cheliceral morphology of the predatory mites (Flechtmann & McMurtry 1992b; Lundgren 2009).

In fact, not all pollens are equally suitable for development and reproduction of polyphagous predacious mites. In the case of Poaceae grasses, the quality of pollen as food source is controversial. Ouyang et al. (1992) described a poor performance of *E. tularensis* when offered *Festuca rubra* L. (Poaceae) or *Hordeum vulgare* L. (Poaceae) pollen, among others, as food. However, Rhodes grass (*Chloris gayana* Kunth) (Poaceae) pollen benefited control of *O. perseae* and *Phyllocoptruta oleivora* (Asmead) by *E. scutalis* (Athias Henriot) in avocados and citrus, respectively, in Israel (Maoz et al. 2009; Palevsky et al. 2010; Maoz et al. 2011) and resulted in larger populations of *E. victoriensis* (Womersley) that controlled populations of eriophyid mites in Australian citrus (Smith & Papacek 1991). In Italian vineyards, Poaceae pollen retained on plant foliage was very important for enhancing phytoseiid populations in natural hedgerows (Duso et al. 2004).

For the above mentioned reasons, the objective of this study was to compare, under semi-field conditions, the effect of two pollen sources, suspected of being qualitatively different, on the efficacy of the specialist *N. californicus* and the generalist *E. stipulatus* on the regulation of *T. urticae* populations.

4.3. Material and Methods

4.3.1. Stock colonies

Tetranychus urticae colonies were maintained on 2-year-old clementine mandarin plants (*C. clementina* cv. Clementina de Nules (INIASEL 22)) grafted on Citrange Carrizo rootstock (*Poncirus trifoliata* (L.) Rafinesque-Schmaltz x *Citrus sinensis* (L.) Osbeck). Plants were not sprayed with insecticides or acaricides. Mite colonies were kept in a glasshouse located at

Instituto Valenciano of Investigaciones Agrarias (IVIA) (Montcada, Valencia, Spain) at 25 ± 1°C, 60 ± 10% RH and natural photoperiod. Colonies were initiated with mites collected in clementine mandarin orchards in the region of La Plana (Castelló de la Plana, Spain).

Individuals of *E. stipulatus* were collected from clementine mandarin orchards infested with *T. urticae* and *P. citri* in Montcada. *Neoseiulus californicus* was originally purchased from Koppert Biological Systems (Spical®). All phytoseiids were maintained on clementine leaves placed upside down on a water-saturated sponge in a plastic tray with water. Strips of wet tissue were placed on the clementine leaf along its periphery to ensure a constant water supply to the phytoseiids and to prevent escape and contamination with other mite species. A small piece of transparent plastic sheet (2 cm^2) folded in the shape of a roof and a few cotton threads placed on each rearing unit provided a shelter and an oviposition substrate for the phytoseiids. Twice a week, *T. urticae* specimens (all stages) were brushed from *T. urticae*-infested clementine leaves into each rearing unit as food. A mixture of *F. arundinacea* and *Carpobrotus edulis* (L.) L. Bolus (Aizoaceae) pollens, a presumably low (Ouyang et al. 1992) and high (Ferragut et al. 1987) quality food sources, respectively, was also offered as food by dusting on the arenas. *Carpobrotus edulis* pollen was obtained from IVIA, where this plant is used as an ornamental. *Festuca arundinacea* pollen was collected from plants cultivated as a ground cover crop in a nearby commercial clementine mandarin orchard. Both pollen types were dried in an oven at 45°C for 24 hours and then sieved through a 75 µm mesh and kept at - 20°C before use. Phytoseiid colonies were maintained in a climate chamber at 25 ± 1 °C, 70 ± 10% RH and 16:8 h L:D photoperiod.

4.3.2. Experimental set-up

Assays were conducted in the glasshouse described above under the same climatic conditions. Cleopatra mandarin (*Citrus reshni* Hort. ex Tan.) rootstock seedlings with approximately 10-15 leaves were used as experimental units as this rootstock is an especially suitable host for *T. urticae* (Bruessow et al. 2010). These plants were grown in 320 ml (8 × 8 × 8 cm) pots. Each plant was isolated by setting the pot on top of a 9 cm diameter Petri dish placed in a round plate (16.5 cm in diameter) filled with water to prevent mite movement among plants. Plants were artificially infested with five adult females of *T. urticae* obtained from the stock colony. To evaluate the effect of pollen provision on the performance of the two predator species included in our study (*E. stipulatus* and *N. californicus*), two consecutive experiments, one for each type of pollen, were considered with five treatments each: 1) prey, 2) predator, 3) predator + prey, 4) predator + pollen, and 5) predator + prey + pollen. In the first experiment, we used *C. edulis* pollen and in the second one, *F. arundinacea* pollen. Eight replicates (= seedlings) per treatment and predator species were considered. Both phytoseiid species were simultaneously tested in each experiment and therefore the "prey" treatment was common for the two predator species.

Ten days after *T. urticae* infestation, when mite colonies were observed in all plants, pollen-treated plants were uniformly dusted with 4 mg of pollen, twice a week without removing the previously application. Subsequently, on those plants receiving a predator treatment, three adult female phytoseiids (3 to 5-day-old) from the laboratory colonies were released. The three females per seedling were deposited on the upper side of different leaves with a fine hair brush.

4.3.3. Population dynamics

Population dynamics were followed at 3-4-day intervals until 100% of citrus seedling leaves were occupied by *T. urticae*. Twice a week, for each plant, one symptomatic (showing characteristic chlorotic spots caused by *T. urticae* feeding) and one asymptomatic leaf (SL and AL, respectively) were randomly selected and monitored. Phytoseiid species and *T. urticae* adult females were counted by the naked eyed. Further, the numbers of SL and AL per plant were counted. At the end of the experiment the entire seedlings were cut and washed in 70% ethanol. Female predatory mites were mounted in Hoyer's medium to confirm the species identity.

4.3.4. Data analysis

Tetranychus urticae and phytoseiid dynamics were analyzed using a Generalized Linear Mixed Model with repeated measurements using IBM® SPSS® statistics, version 19.0.0 (SPSS Inc 2010). Treatment was considered as a fixed factor and day as a random one. When significant differences were found, pair-wise comparisons of the fixed factor levels were performed with the least significant difference (LSD) post hoc test ($P < 0.05$). When required, mite numbers were log transformed to fulfill the assumption of normality. Different covariance structures were evaluated and Akaike information criterion (AIC) (Akaike 1974) was used to select the most parsimonious model. When significant differences were found ($P < 0.05$), efficacies were calculated using Abbott's formula (Abbott 1925). Data on the percentage of symptomatic leaves were subjected to one-way analysis of variance (ANOVA) together with a LSD test for mean separation ($P < 0.05$). When required, percentage data were subjected to the angular transformation to fulfill the assumptions of normality and homogeneity of variance. When significant differences were found ($P < 0.05$), efficacies were calculated using Abbott's formula. Two-sample comparison was used to compare SL and *T. urticae* reduction between pollen types for each phytoseiid species. A Poisson distribution was appropriate for phytoseiid numbers.

4.4. Results

4.4.1. *Tetranychus urticae* dynamics and damage

In the *C. edulis* experiment, the five *T. urticae* females released caused 100% leaf occupation at day 16 (26 days after the initial *T. urticae* infestation) in those treatments where phytoseiids were not released. Likewise, that day only 1 out of 8 replicates for the *T. urticae* treatment had not reached 100% occupation in the *F. arundinacea* treatment and therefore we proceeded with the final destructive sampling as well. At that day, both phytoseiid species had significantly reduced the percentage of SL when compared with the *T. urticae* control treatment, independently of pollen supply and quality (Table 4.1). Only in the case of *N. californicus* fed with *F. arundinacea* pollen, did pollen supply significantly reduce the percentage of SL relative to the predator treatment without pollen ($P = 0.04$) (Table 4.1). However, efficacies were different for each species and pollen type. *Neoseiulus californicus* reduced SL by 76 and 40% when offered *F. arundinacea* and *C. edulis* pollen, respectively (*W* test = 46.0; $P = 0.007$) (Fig. 4.1 a). Similarly, *E. stipulatus* reduced SL by 27 and 46%, respectively (*t* test = 2.42; $P = 0.032$) (Fig. 4.1 b). These decreases were also observed when *T. urticae* populations were scored (Fig. 4.2). In both experiments, the two phytoseiids were able to successfully reduce spider mite populations but their effect was independent of pollen provision and quality (Table 4.2). In this case, reductions were 97 and 86% for *N. californicus* when offered *F. arundinacea* and *C. edulis* pollen, respectively. Similarly, *E. stipulatus* reduced *T. urticae* populations a 72 and a 75%, respectively. However, for each species, these differences were not significant (*N. californicus*: *t* test = 0.16; $P = 0.876$; *E. stipulatus*: *t* test = -0.94; $P = 0.362$) (Fig 4.3).

Table 4.1. *P*-values for treatment comparison of plant damage level (measured as number of symptomatic leaves per plant) within each type of pollen and for each phytoseiid species at day 16 (ANOVA).

Type of pollen	Treatment	*N. californicus*	*E. stipulatus*
F. arundinacea	Prey *vs* Prey + predator	0.0001	<0.0001
	Prey *vs* Prey + predator + pollen	0.0001	0.0010
	Prey + Predator *vs* Prey + predator + pollen	0.0410	0.9490
C. edulis	Prey *vs* Prey + predator	0.0010	0.0001
	Prey *vs* Prey + predator + pollen	0.0020	0.0001
	Prey + Predator *vs* Prey + predator + pollen	0.7340	0.4250

Table 4.2. *P*-values for treatment comparison of *T. urticae* numbers within each type of pollen and for each phytoseiid species along the experiment (GLMM).

Type of pollen	Treatment	*N. californicus*	*E. stipulatus*
F. arundinacea	Prey *vs* Prey + predator	0.0001	0.0100
	Prey *vs* Prey + predator + pollen	0.0001	0.0001
	Prey + Predator *vs* Prey + predator + pollen	0.5840	0.1540
C. edulis	Prey *vs* Prey + predator	0.0001	0.0001
	Prey *vs* Prey + predator + pollen	0.0001	0.0001
	Prey + Predator *vs* Prey + predator + pollen	0.9250	0.5660

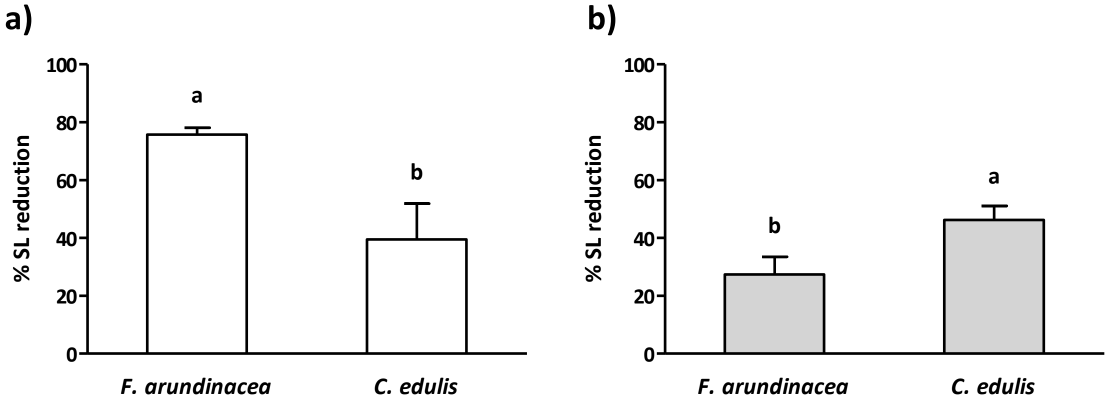

Fig. 4.1. Reduction (%) of plant damage level at day 16 (measured as number of symptomatic leaves per plant, SL) by *N. californicus* (a) and *E. stipulatus* (b) supplied with different types of pollen. Different letters above the bars indicate significant differences ($P < 0.05$; LSD test) between pollen types.

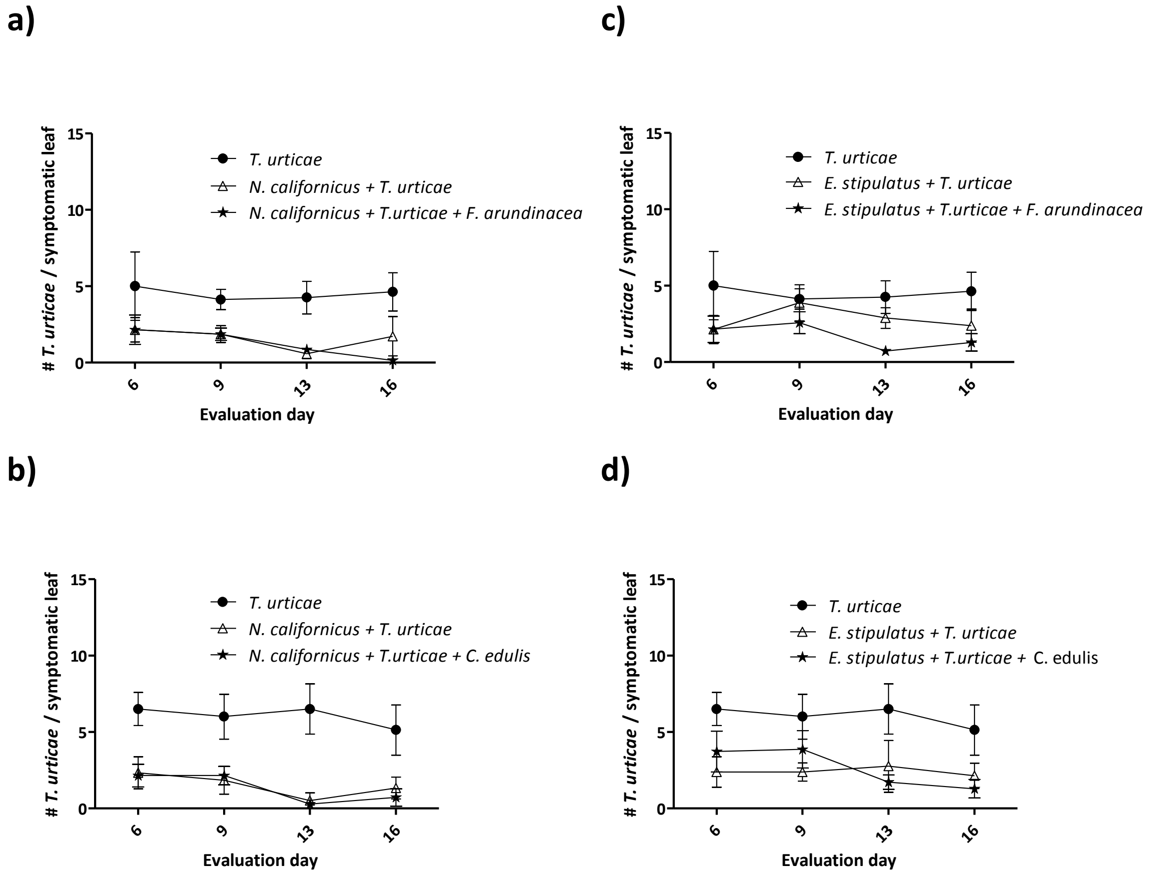

Fig. 4.2. Population dynamics of *T. urticae* (mean number of mites ± SE) on leaves of Cleopatra mandarin rootstock seedlings in presence of *N. californicus*, *E. stipulatus* or no phytoseiids, with and without pollen supply of *F. arundinacea* (a, c) or *C. edulis* (b, d).

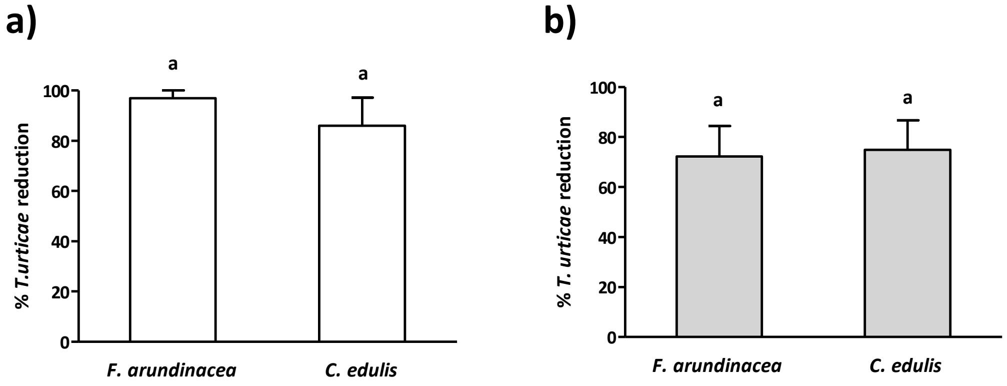

Fig. 4.3. Reduction (%) of *T. urticae* numbers at day 16 by *N. californicus* (a) and *E. stipulatus* (b) supplied with different types of pollen. Different letters above the bars indicate significant differences ($P < 0.05$; LSD test) between pollen types.

4.4.2. Phytoseiid dynamics

Phytoseiid abundance and dynamics was different for each species and pollen type considered (Figs. 4.4 and 4.5). In the case of *N. californicus* on SL (Figs. 4.4 a and b), no population increase was observed when added *F. arundinacea* pollen ($F_{1,\ 54} = 0.400$; $P = 0.530$), whereas the addition of *C. edulis* effectively increased population numbers (no statistical test was applied in this case because just one phytoseiid was found in the prey + predator treatment). In the case of *E. stipulatus* (Figs. 4.4 c and d), the addition of both types of pollen significantly increased its numbers ($F_{1,\ 58} = 57.138$; $P < 0.0001$ for *C. edulis* pollen; no statistical test was performed for *F. arundinacea* because just one individual was obtained). When these increases obtained were compared, the addition of *C. edulis* resulted in a 5-fold population increase compared to *F. arundinacea* pollen. When considering AL, similar phytoseiid dynamics and abundances were observed.

In no-prey no-pollen treatments, no phytoseiids were found during the samplings and this is indicative that either pollen or prey is necessary for survival (Fig. 4.5). *Festuca arundinacea* pollen addition allowed *E. stipulatus* and *N. californicus* to survive (Figs. 4.5 a and c). However, when *E. stipulatus* was supplied with *C. edulis* pollen a 40-fold population increase was observed when comparing to *F. arundinacea* (Fig. 4.5 d) and this increase was higher than that observed when the prey was added (Fig. 4.4 d). Contrarily, *N. californicus* exhibited lower numbers when supplied with only pollen of either *F. arundinacea* or *C. edulis* (Figs. 4.5 a and b) compared to the increases observed when pollen was offered with prey (Figs. 4.4 a and b).

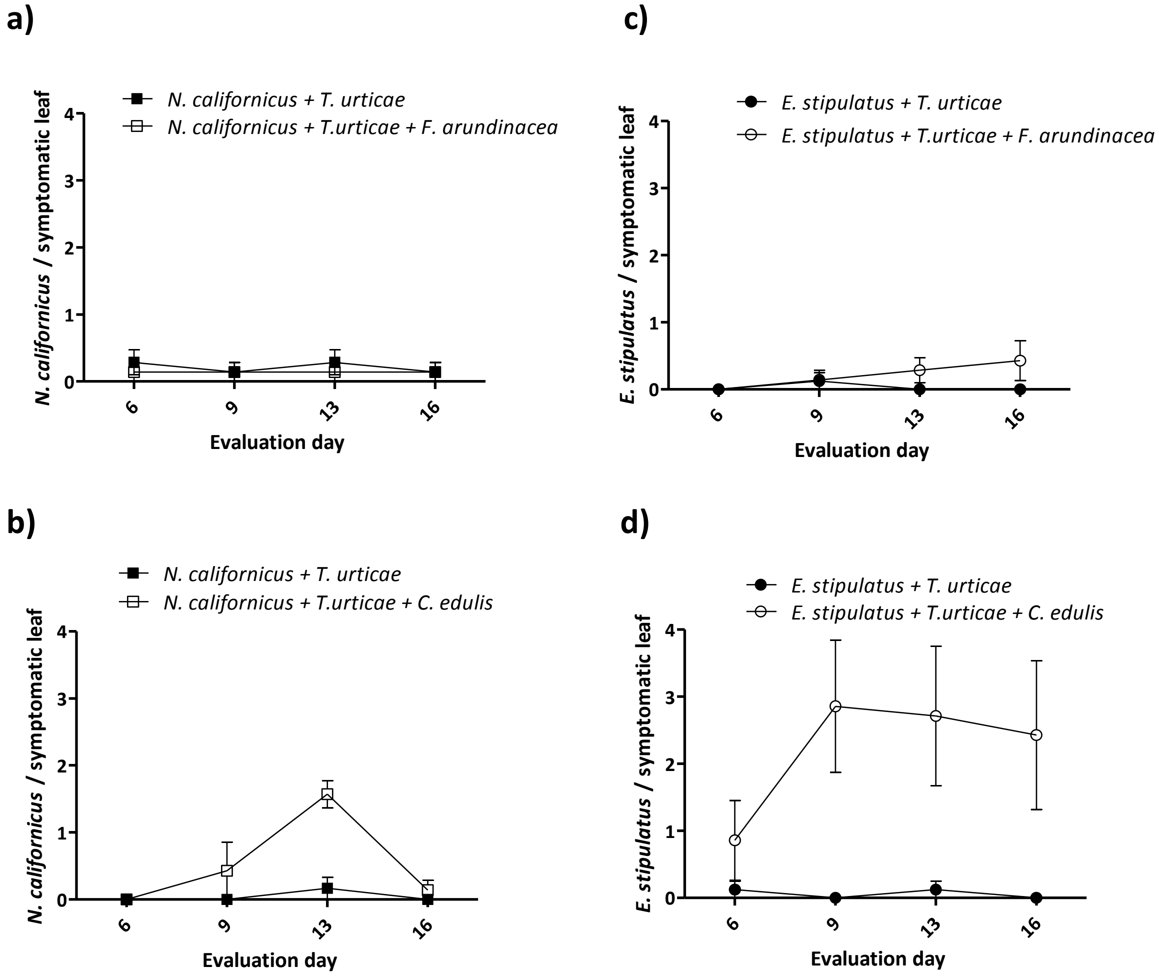

Fig. 4.4. Population dynamics (mean number of phytoseiids ± SE) of *N. californicus* (a, b) and *E. stipulatus* (c, d) on symptomatic leaves (SL) of Cleopatra mandarin rootstock seedlings with and without pollen supply of *F. arundinacea* (a, c) or *C. edulis* (b, d).

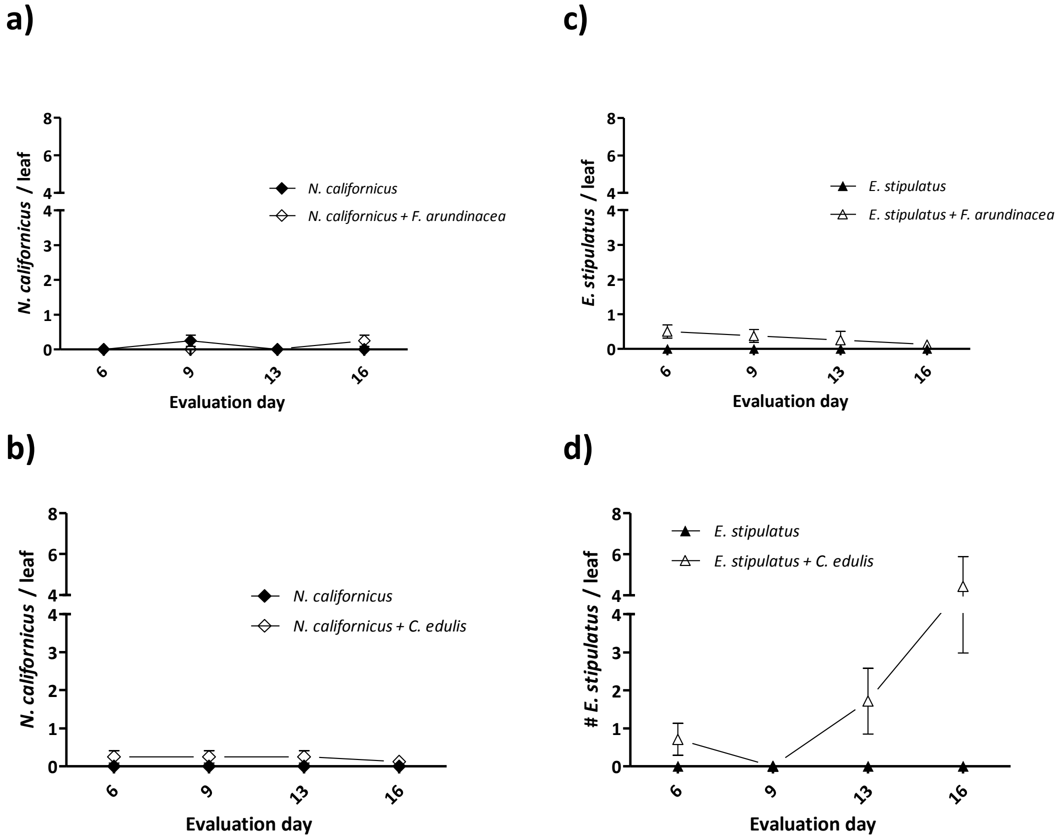

Fig. 4.5. Population dynamics (mean number of phytoseiids ± SE) of *N. californicus* (a, b) and *E. stipulatus* (c, d) on leaves of Cleopatra mandarin rootstock seedlings with and without pollen supply of *F. arundinacea* (a, c) and *C. edulis* (b, d).

4.5. Discussion

McMurtry and Scriven (1966a; 1966b) were the first to show that biological control of phytophagous mites could be enhanced by the presence of pollen. These authors found that *Euseius hibisci* consumed fewer prey (*Oligonychus punicae* (Hirst)) per individual when fed with a mixed diet of pollen and prey but had a higher reproductive rate that implied a higher intensity of predation. In our study, the provision of pollen as alternative food did not enhance the ability of *N. californicus* and *E. stipulatus* to reduce populations of *T. urticae*. However, efficacy in *T. urticae* population reduction and consequently in damage reduction, was observed in all cases where phytoseiids were present suggesting that both species contributed to reduce pest numbers. Nevertheless, their efficacy depended on the phytoseiid species considered: *N. californicus* was a more efficient predator than *E. stipulatus* with and without pollen and independently of the type of pollen supplied.

Differences found not only in pest numbers reduction but also in the dynamics and abundance of predatory mites could be attributed to their different life-style, as suggested by McMurtry and Croft (1997) and Croft et al. (1998; 2004).

Neoseiulus californicus is considered a good natural enemy of *T. urticae* (Abad-Moyano et al. 2010a) and in our assays it effectively reduced *T. urticae* numbers and its damage irrespective of pollen supply. *Neoseiulus californicus* behaves as a specialist predator of tetranychid mites (McMurtry & Croft 1997; Croft et al. 1998; Croft et al. 2004). However, it can also alternatively feed on pollen to survive and even reproduce with this non-prey food item when the prey is scarce or absent (Castagnoli & Simoni 1999; Sazo et al. 2006; Ragusa et al. 2009). Our results are in agreement with these observations and pollen supply ensured the maintenance of *N. californicus* when no prey was available (Figs. 4.5a and 4.5b), even in the case of *F. arundinacea* pollen, a food source that has proved not to be as profitable as other pollen types (Sazo et al. 2006; Ragusa et al. 2009; González-Fernández et al. 2009). However, population increases were only observed in prey-treatments with *C. edulis* pollen suggesting that this pollen has a positive effect in population increases but only if prey is present.

Several papers have discussed the role of *E. stipulatus* as a natural enemy of *T. urticae* (Ferragut et al. 1987; Grafton-Cardwell et al. 1997; Abad-Moyano et al. 2009a; Abad-Moyano et al. 2009b; Abad-Moyano et al. 2010b). In this study, significant reductions in tetranychid numbers and damage were observed for this phytoseiid either with or without pollen addition. Interestingly, extreme population increases observed with *C. edulis* pollen did not result in higher efficacy. The genus *Euseius* is considered a pollen feeder/generalist predator (McMurtry & Croft 1997; Croft et al. 1998; Croft et al. 2004). Its ability to feed on and take profit from certain types of pollen can be the result of adaptations in morphology (e.g. feeding apparatus, sensory organs), physiology (digestive system) and behavior (e.g. feeding preferences) (Flechtmann & McMurtry 1992a; Flechtmann & McMurtry 1992b; van Rijn & Tanigoshi 1999) relative to other phytoseiid genera. One aspect to consider when evaluating the value of different pollen types as a food source is the rate of oviposition, and therefore the intrinsic rate of population increase when females have been feeding for a fixed period on this food source (van Rijn & Tanigoshi 1999). In our case, both types of pollen rendered a clearly different effect on *E. stipulatus* populations suggesting qualitative differences. On the one hand, *F. arundinacea* pollen was not as suitable as that of *C. edulis* for maintaining high and durable populations of *E. stipulatus* despite the presence of the tetranychid prey as alternative food. This result is in agreement with that obtained by Ouyang et al. (1992). These authors demonstrated that pollen from most grasses, including that of *Festuca rubra*, provide sufficient nutrition to *E. tularensis* to develop and reproduce normally during at least one generation but during the following generation a bottleneck in survival appeared suggesting that grass pollen alone did not provide a sufficient long-term food source. However, even if not entirely suitable, *F. arundinacea* pollen was necessary and good enough to maintain a stable phytoseiid population (Fig. 4.5c). Remarkably, *E. stipulatus* numbers were even higher in absence of prey (Fig. 4.5d) and this result suggests a possible negative effect of *T. urticae* on *E. stipulatus* dynamics. On the other hand, the maximum number of *E. stipulatus* attained per leaf was clearly different depending on the pollen source. This number was below one for *F. arundinacea* pollen and up to 7 individuals (in asymptomatic leaves, data not shown) when offered *C. edulis* pollen.

Schausberger (2003) described that for a diet to serve as a viable alternative food source, it must be able to maintain phytoseiid mites over the long term rather than increase the capacity for egg production because an over-abundance of phytoseiid mites can lead to an over-consumption of the food resource and may cause an increase in the frequency of cannibalism. In this logic, neither *F. arundinacea,* nor *C. edulis* are viable alternative food sources for the pollen feeder *E. stipulatus*. Firstly, *F. arundinacea* pollen cannot maintain long-lasting populations (Fig. 4.5c). Secondly, *C. edulis* pollen increases their populations so quickly (40-fold increase, when compared with only *F. arundinacea* pollen) (Fig. 4.5d) that it could easily lead to the occurrence of either cannibalism or lethal and non lethal intraguild interactions with other phytoseiid species as observed with *N. californicus* and *P. persimilis* (Abad-Moyano et al. 2010b; Abad-Moyano et al. 2010c). In fact, no significant increases in efficacy were observed in correspondence with *E. stipulatus* enormous population increases. In another study (Pérez-Sayas et al., in preparation), we investigated phytoseiid gut content in field conditions using molecular techniques, and we have observed that *E. stipulatus*, even when *T. urticae* is abundant, preferentially feeds on other food sources. Intraguild predators, including other phytoseiid species, could be among these sources.

Because *E. stipulatus* population increases when offered pollen (Figs. 4.5c and 4.5d) were almost the same as when offered pollen and prey (Figs. 4.4c and 4.4d), we can conclude that *E. stipulatus* increases were related to pollen supply only. Indeed, when *T. urticae* was offered as the solely food source, *E. stipulatus* showed a poor performance (Figs. 4.4c and 4.4d) as previously described in laboratory conditions by other authors (Ferragut et al. 1987; Zhimo & McMurtry 1990; Bouras & Papadoulis 2005; Abad-Moyano et al. 2009b).

McMurtry (1992) proposed that species with broad prey ranges (omnivorous and polyphagous) may remain longer on plants and regulate pest mite outbreaks effectively preying on supplemental foods as pollen when prey mites are not available. Therefore, pollen could contribute and enhance stability of the predator populations during periods of low spider mite populations. But its effect, as shown in this study, could be different depending on the type of pollen and phytoseiid species considered. *Festuca arundinacea* has resulted to provide low quality pollen (when compared with *C. edulis* pollen) which is good enough to maintain low phytoseiid populations of *E. stipulatus* and *N. californicus* in periods of food scarcity. However, the poor quality of this pollen may prevent pollen feeders (*E. stipulatus*) from reaching explosive numbers in the field, as observed with *C. edulis* (a high quality pollen for *E. stipulatus*), and therefore this effect could benefit specific phytoseiid species suffering the intraguild predation by *E. stipulatus*. Furthermore, while *F. arundinacea* pollen alone does not appear to support long-term survival of phytoseiids, *F. arundinacea* cover crop may still be useful as it can provide other food sources as thrips (Aguilar-Fenollosa et al., in preparation), frequently used by *N. californicus* (Rodriguez et al. 1992; Baal et al. 2007).

Thus, not only the phenology of the ground cover (Aguilar-Fenollosa et al. 2011b) but also the quality of the food source provided (pollen and others) by this ground cover could have a serious impact on phytoseiid dynamics and abundance, and therefore in pest control. In this logic, results obtained with *F. arundinacea* in this study provide at least one plausible explanation for the field results obtained by Aguilar-Fenollosa et al. (2011a; 2011b) and reassure that *F. arundinacea* is a cover crop that should be encouraged in citrus orchards.

CAPÍTULO 5

SPATIAL DISTRIBUTION OF TWO PHYTOSEIID MITES *Phytoseiulus persimilis* AND *Neoseiulus californicus* IN CLEMENTINE YOUNG PLANTS

5.1. Abstract

Augmentative biological control through augmentative releases is a common practice against some citrus pests such as *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), *Planoccocus citri* Risso (Hemiptera: Pseudococcidae), and is developing in other species such as *Tetranychus urticae*. In the case of the *T. urticae*, releases of the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus* resulted successful in nurseries. However, under field conditions, phytoseiids are not always detected after releases and control of *T. urticae* has not always been satisfactory, especially in the case of *N. californicus*. There is no information on the behavior of these phytoseiid mites in citrus orchards. Understanding their dispersal and preying activity could be useful to improve augmentative releases. Results showed that the phytoseiids exhibited a similar spatial distribution pattern under different prey densities. Both phytoseiids were predominantly found on leaves, where *T. urticae* colonies were present. However, at low pest densities, *N. californicus* showed a higher tendency to move to the trunk. Furthermore, prey detection by molecular methods proved *P. persimilis* to be a superior predator, even at lower prey densities. Based on these results, hot spot augmentative releases of *P. persimilis* are recommended.

Key words: distribution pattern, Phytoseiids, citrus

5.2. Introduction

Tetranychus urticae Koch (Acari: Tetranychidae) is a key pest of citrus orchards in Spain, especially clementines (Martínez-Ferrer et al. 2006; Jacas et al. 2010; Aguilar-Fenollosa et al. 2012). The control of this pest is mainly based on the use of pesticides; however, this strategy is associated with problems such as pesticide resistance and other environmental hazards. Currently, efforts are been directed to the identification of biorational more sustainable control methods as biological control.

Augmentative biological control against different citrus pests is becoming increasingly important in citrus IPM in Spain (Jacas & Urbaneja 2010) and elsewhere (Meagher & French 2004; van Driesche et al. 2009). This is a common practice to control some citrus pests such as the the California red scale *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) (Jacas & Urbaneja 2010) or the mealybug *Planoccocus citri* Risso (Hemiptera: Pseudococcidae) (Olivero et al. 2003; Martínez-Ferrer 2003). In the case of *T. urticae*, augmentative releases with the predatory mites *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) have proved successful in different horticultural crops (Helle & Sabelis 1985a; Van Lenteren & Woets 1988; van Lenteren 2000). Releases of both predatory mite species in citrus nurseries were effective in reducing *T. urticae* infestations (Grafton-Cardwell et al. 1997; Abad-Moyano et al. 2010a). Furthermore, the combination of *P. persimilis* releases with the neonicotinoid insecticide imidacloprid applied as a drench was highly effective for the management of the main pests in Spanish clementine nurseries (Argolo et al. 2012). Under field conditions, releases of *N. californicus* and *P. persimilis* in citrus have yielded, in general, satisfactory results. Intriguingly, individuals of these species could not be recovered later in the release trees following the standard monitoring systems for predatory mites in citrus (Urbaneja et al. 2012). As a consequence, these standard methods originally developed to estimate the densities of the most widespread phytoseiid mite in citrus orchards, *Euseius stipulatus* (Athias-Henriot), are considered not suitable to estimate densities of *N. californicus* and *P. persimilis*.

Prey patchiness and density are key factors to take into account to optimize the release strategy to enhance efficacy as much as possible. *Tetranychus urticae* exhibits rapid population growth and has been widely demonstrated to display an aggregative distribution pattern (Raworth 1986; So 1991; Greco et al. 1999; Liu et al. 2002; Yasuyuki et al. 2004; Ahmadi et al. 2005; Grbic et al. 2007). *Phytoseiulus persimilis* and *N. californicus* have distinct life-styles (McMurtry & Croft 1997) and different responses to prey density and patchiness (Escudero & Ferragut 1996; Pratt 1998). *Phytoseiulus persimilis* is a specialist predator of *Tetranychus* spp. and has shown high ability to quickly find and exploit new prey patches (Skirvin & Fenlon 2003; Walzer et al. 2009; Alatawi et al. 2011). During the foraging process, this species has a higher tendency to disperse from one plant to another rather than staying in the same plant. However, under high prey availability, the dispersal rate may decrease and *P. persimilis* may spend more time feeding on prey than moving (Gontijo et al. 2012). *Neoseiulus californicus* is considered a generalist predator of tetranychid species that preferentially moves within-plant rather than moving from plant to plant due to its ability to feed on alternative prey and non-prey food sources (e.g. pollen, fungal spores, etc.). Therefore, *N. californicus* is less dependent on the ability to detect new prey patches (McMurtry & Croft 1997; Greco et al. 1999). Besides prey distribution and density, plant architecture can also plant architecture can also influence the foraging efficiency of predatory mites. Gontijo et al (2010) reported that the level of complexity of the plant architecture may indirectly influence natural enemies through offering spatial refuges to the herbivores and predators (Onzo et al. 2003) or modifying their distribution (Clark & Messina 1998) and abundance (Legrand & Barbosa 2003). The spatial distribution of *N. californicus* and *P. persimilis,* separately or together, has been studied on different host-plants with marked distinct architecture from citrus as strawberries, cucumbers, dwarf hops and beans (Pratt 1998; Greco et al. 1999; Auger et al. 1999; Lilley 1999; Khalequzzaman et al. 2007; Fitzgerald et al. 2008; Gómez-Moya & Ferragut 2009; Walzer et al. 2009; Gontijo et al. 2010). Furthermore, whether different *T. urticae* densities may modulate their dispersal or starving behavior, as observed by Escudero and Ferragut (1996) in beans, has not been addressed for citrus either. Understanding the dispersal of predatory mites and their preying activity on their tetranychid preys can be useful information to improve augmentative releases. Therefore, the aim of this work has been to assess the spatial distribution of *P. persimilis* and *N. californicus* on clementine plants infested with *T. urticae* and to relate it to infestation levels and predator feeding status.

5.3. Material and Methods

5.3.1. Stock cultures

Tetranychus urticae colonies were obtained from mites collected in clementine mandarin orchards in the region of La Plana (Castelló de la Plana, Spain). These colonies, which were later used in our assays, were maintained on lemon fruits in a climatic chamber at 25 ± 1°C, 70-80% RH and 16:8 h (L:D) photoperiod. Lemons were collected from a pesticide-free orchard located at Valencian Institute of Agricultural Research (IVIA) (Montcada, Valencia, Spain).

Neoseiulus californicus and *P. persimilis* individuals were obtained from the commercial product (Spical® and Spidex®, respectively) from Koppert Biological Systems S.L.

5.3.2. Plant material

Two-year-old clementine mandarin plants (*Citrus reticulata* Blanco [Clementina de Nules cultivar Iniasel 22] grafted on Citrange Carrizo rootstock [*Poncirus trifoliata* (L.) Rafinesque-Schmaltz x *Citrus sinensis* (L.) Osbeck]) were used in our assays. One month before the starting of the experiment, plants were pruned and defoliated and kept in a glass greenhouse located at IVIA at 22 ± 2°C, 55 ± 10% RH and under natural photoperiod. When plants had around 150 young fully-expanded leaves, they were used for the assays.

5.3.3. Experimental procedure

The experiment was repeated twice in summer and late autumn 2011. In each assay, the spatial distribution of *N. californicus* and *P. persimilis* in clementine mandarin plants infested with *T. urticae* was assessed every 5 hours along an experimental period of 25 hours. Simultaneously, whether these predators had preyed on *T. urticae*, was ascertained by use of molecular methods.

The experimental unit consisted of 20 plants distributed in a regular grid of 5 groups of 4 plants each (=replicate) per phytoseiid species. Both phytoseiid species were simultaneously tested in each experiment. Each species was kept in separate benches inside the same greenhouse. Additionally, plants were placed on the top of a brick inside a plastic tray full of water to avoid mite movement between neighbouring plants. Assays were conducted in the greenhouse described above under the same climatic conditions.

Plants were artificially infested with 10 *T. urticae* adult females obtained from the stock colony. These mites were placed with the aid of a fine brush on the upper side of 10 different leaves randomly chosen. Thirteen days after *T. urticae* infestation, when mite colonies were observed in all plants, a previous sampling was performed to assess mite infestation. This sampling consisted of counting the number of *T. urticae* adult females in six symptomatic leaves (SL) (those with chlorotic spots on the upper side of the leaf) per plant and species. Subsequently, 50 phytoseiid mites either, *N. californicus* or *P. persimilis*, were released per plant on the upper side of different leaves randomly chosen, with a fine brush. Phytoseiids were directly taken from their respective commercial containers.

To determine the spatial distribution of both phytoseiids on three different plant strata (leaves, branches and trunk), a destructive sampling was conducted every 5 hours for a period of 25 hours. Four trees per phytoseiid species were sampled at once. The destructive sampling consisted of the section of each part of the plant: leaves, branches and trunk, which were deposited on separate plastic containers containing 70% ethanol. To monitor mite movement during each 5-hour interval, mites crawling up or down tree trunks were captured on 8 cm wide both-sides sticky yellow bands (Koppert Biological Systems S.L.). These bands were set 40 cm above soil level and were installed at 5 hour intervals, starting with the first group of 4 trees that had to be sampled 5 hours later when phytoseiids were initially released and so on. As a result, the sticky band was held in place for the 5 hours prior to sampling only. During the destructive sampling, the sticky band and the piece of trunk that was surrounding it were placed on a 15 cm diameter Petri dish. Both plastic containers and Petri dishes were taken to the laboratory for evaluation. All phytoseiids in ethanol were initially counted and later digested in lactic acid and mounted in Hoyer's medium for species identification. Phytoseiids on the adhesive band and in the piece of the trunk that was surrounding it (hereafter, trunk + band) were also counted, isolated in Eppendorf tubes and then frozen at -80°C.

To determine the contribution of the released phytoseiid on *T. urticae* control, the first five live phytoseiids found on the leaves of each plant during the destructive sampling were individually isolated in Eppendorf tubes and immediately frozen with liquid nitrogen. Afterwards, the Eppendorf tubes were transferred to crioboxes and conserved at -80°C for later DNA extraction and molecular identification. In total, 20 phytoseiids per sampling time were obtained per species and treatment (100 in total). Additionally, 5 phytoseiids directly obtained from the commercial container were frozen in the same way at the beginning of the experiment and used as control.

5.3.4. Molecular procedure

5.3.5. DNA extraction and molecular identification

Mite DNA was isolated following the "Salting out" protocol (Sunnucks & Hales 1996). DNA samples were kept at -20°C for the molecular analysis. Molecular identification was done using a multiplex PCR developed by Pérez-Sayas et al. (2012) that allows to identify two tetranychid preys (*T. urticae* and *P. citri*) and six phytoseiids predators (*Euseius stipulatus* (Athias-Henriot), *Phytoseiulus persimilis*, *Neoseiulus californicus*, *Typhlodromus phialatus* Athias-Henriot*, Amblyseius barkeri* (Hughes) and *Amblyseius swirski* (Athias-Henriot) in a single amplification reaction. Multiplex-PCR amplification conditions were performed as described by reaction was adjusted according to a final volume of 12.5 µl (Pina et al. 2011; Pérez-Sayas et al. 2012). Amplification profile was: one denaturation step at 94°C for 4 min, 35 cycles at 92°C for 30 s, 55°C for 30 s, followed by an extension at 72°C for 30 s and a final extension at 72°C for 10 min. Amplification was performed in a Bio-Rad MJ Research Thermal Cycler PTC-100®. Fragment sizes were analyzed in an ABI/PE 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) at the SCSIE Sequencing facility (Universitat de València, Spain). Fragment length lecture was performed using Peak Scanner™ Software v1.0 (Applied Biosystems, 2006). Positive prey detection was considered when a peak was observed on the corresponding size to the amplified fragment of *T. urticae* (121 base pairs). Detectability half-life (post-ingestion time during which 50% of positives were still detectable) of *T. urticae* in *P. persimilis* and *N. californicus* were 3 hours and 13 hours, respectively (Pina et al. 2011).

5.3.6. Data analysis

One-way analysis of variance (ANOVA) followed by Tukey test for mean separation ($P < 0.05$) was used to compare the initial numbers of *T. urticae* adult females per leaf/plant before predator release. Comparisons were made for each assay between plants that received different phytoseiid species (i.e. assay 1: plants with *P. persimilis* vs. plants with *N.*

californicus) and for plants where the same phytoseiid species was released between assays (i.e. plants with *P. persimilis*: assay 1 *vs.* assay 2). When required, data were subjected to log transformation to fulfill the assumptions of normality and homogeneity of variance.

For each phytoseiid species, the spatial distribution was analyzed using a Generalized Linear Mixed Model (GLMM) using IBM® SPSS® statistics, version 19.0.0 (SPSS Inc. IBM Company, 2010). Substrate (leaf, branches and trunk) was considered as fixed factor and time as a random one. When significant differences were found, pair-wise comparisons of the fixed factor levels were performed with the least significant difference (LSD) post hoc test ($P < 0.05$). Akaike information criterion (AIC; Akaike (1974)) was used to select the most parsimonious model.

Regression lines for the percentage of phytoseiids that disappeared during the sampling in each assay were compared using Statgraphics Plus 5.0. Assay number, time and their interaction were considered in the analysis. When no significant differences in slope and intercept were observed between assays for each species, parallelism and equal intercept were forced. Molecular prey detection through assays and time and their interaction was initially analyzed with hierarchical loglinear models. However, no significant models were obtained. Therefore, prey detection was analyzed with contingency tables. Fisher's Exact Test for count data was used as statistical test (R version 2.15.0 (2012-03-30)). Phytoseiid species were analyzed separately, first comparing proportions between times for each assay, later between assays for each time, and finally between substrates within each assay. When no significant differences were obtained for these comparisons, total percentages per species were analyzed using the same test.

5.4. Results

5.4.1. *Tetranychus urticae* density

The initial *T. urticae* sampling carried out in six symptomatic leaves per clementine plant in the first assay rendered a mean of 2.9 and 2.0 adult females per symptomatic leaf (SL) in the plants that later received *P. persimilis* and *N. californicus*, respectively. In the second assay, a mean of 0.2 and 0.6 adult females per SL was recorded in those plants where *P. persimilis* and *N. californicus* were later released, respectively. When *T. urticae* initial densities in the same assay were compared, no differences were found between plants (*T. urticae* densities*:* Assay 1: $P = 0.169$; Assay 2: $P = 0.822$). However, *T. urticae* densities were different for the different assays irrespective of the phytoseiid species to be released later (*P. persimilis* releases: Assay 1 *vs*. Assay2: $P < 0.001$; *N. californicus* releases: Assay 1 *vs.* Assay 2: $P = 0.007$). The initial density of *T. urticae* was 6.1 times higher in assay 1 than in assay 2.

5.4.2. Spatial distribution

Spatial distribution of *P. persimilis* and *N. californicus* in both assays is shown in Figs. 5.1 and 5.2. *Phytoseiulus persimilis* was significantly more abundant in leaves than on branches and trunk, during all the experimental period in both assays independently of prey density (assay 1: $F = 453.43$, $df = 2, 54$ $P < 0.001$; assay 2: $F = 907.65$, $df = 2, 57$, $P < 0.001$) (Figure 5.1a, b). For both assays, most phytoseiids (≈ 90%) were found on leaves, whereas around 6.5% and 3.5% phytoseiids were found on trunk and branches, respectively (Figure 5.1c, d).There were no significant differences among these two strata in both assays (assay 1: leaves *vs*. trunk: $t_{54} = 1.35$; $P = 0.183$; assay 2: leaves *vs*. trunk: $t_{57} = 0.02$; $P = 0.981$).

Similarly, *N. californicus* was mostly found on leaves followed by trunk and branches and this distribution was consistent in both assays (Figure 5.2a, b). In assay 1, 78% of individuals were found on leaves, 5% on branches and 17% on trunk, with significant differences among substrates (assay 1: $F= 276.06$, $df = 2, 51$ $P < 0.001$) (Figure 5.2c). In assay 2, *N. californicus* was again significantly more abundant on leaves (59%) than on other substrates. However, a significant increase of phytoseiids on trunk was observed relative to assay 1 (20 % increase; assay 2: $F= 141.79$, $df = 2, 57$, $P < 0.001$) (Figure 5.2d).

The overall percentage of phytoseiids that disappeared along the experiment was different depending on the species. For *N. californicus*, no differences were found neither between assays (intercept, $P = 0.615$) nor between time (slope, $P = 0.321$), so equal slopes and intercepts were forced. A total of 2.3 ± 1.3 *N. californicus* disappeared per sampling interval (5 hours). For *P. persimilis*, equal slopes were found between assays ($P = 0.276$). Differences in intercept ($P < 0.001$) were attributed to a probably different quality of the commercial phytoseiids used because the only difference between assays was found for the first 5-hours interval. For this species, the disappearance rate per sampling interval was 6.3 ± 1.2. The tendency to disappear was 2.7-fold higher in *P. persimilis* than in *N. californicus*.

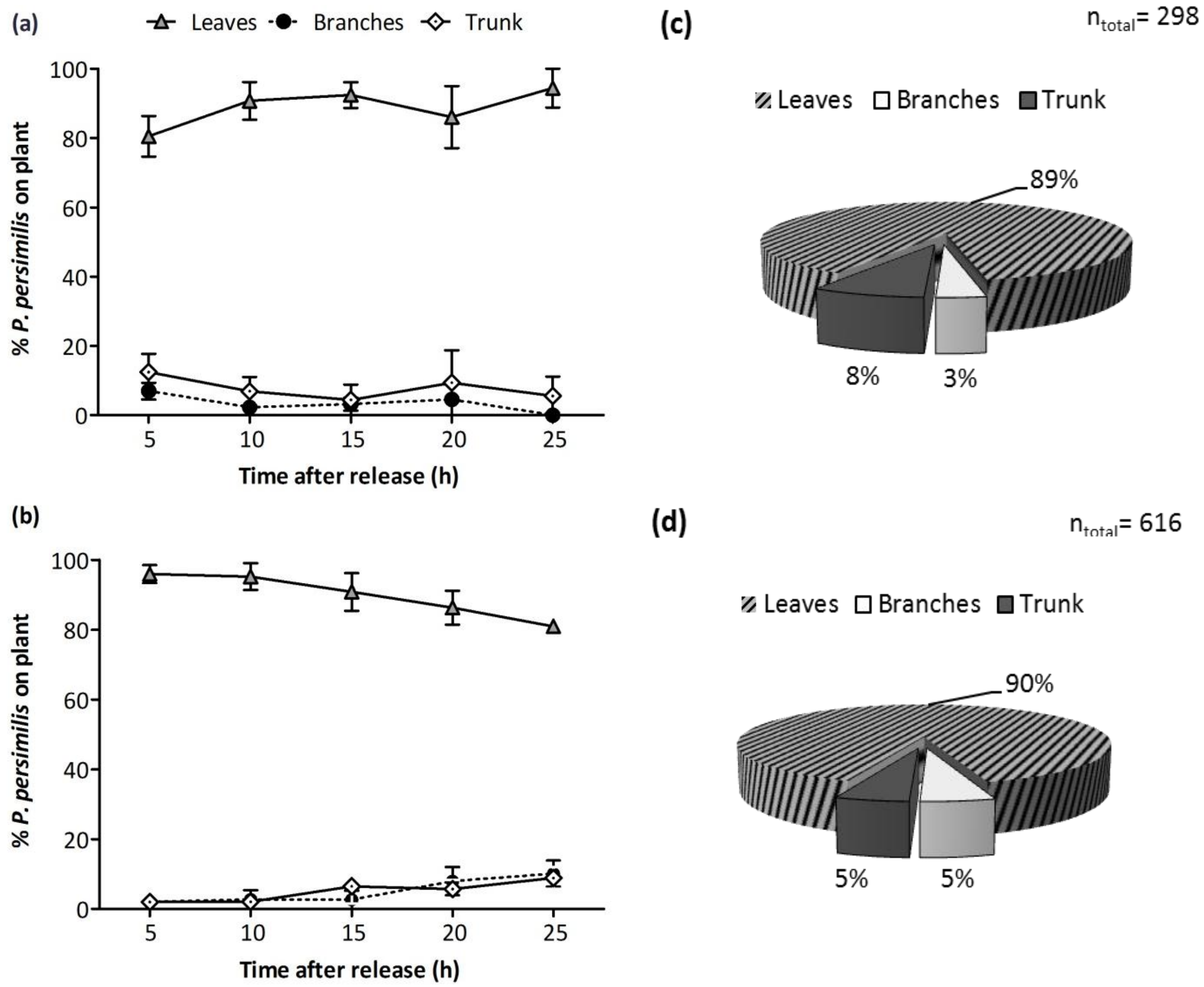

Fig. 5.1. Population dynamics (mean and SE, in %; a and b) and distribution (%) (c and d) of *P. persimilis* in the three different substrates in both assays (assay 1: a, c; assay 2, b, d) on clementine plants.

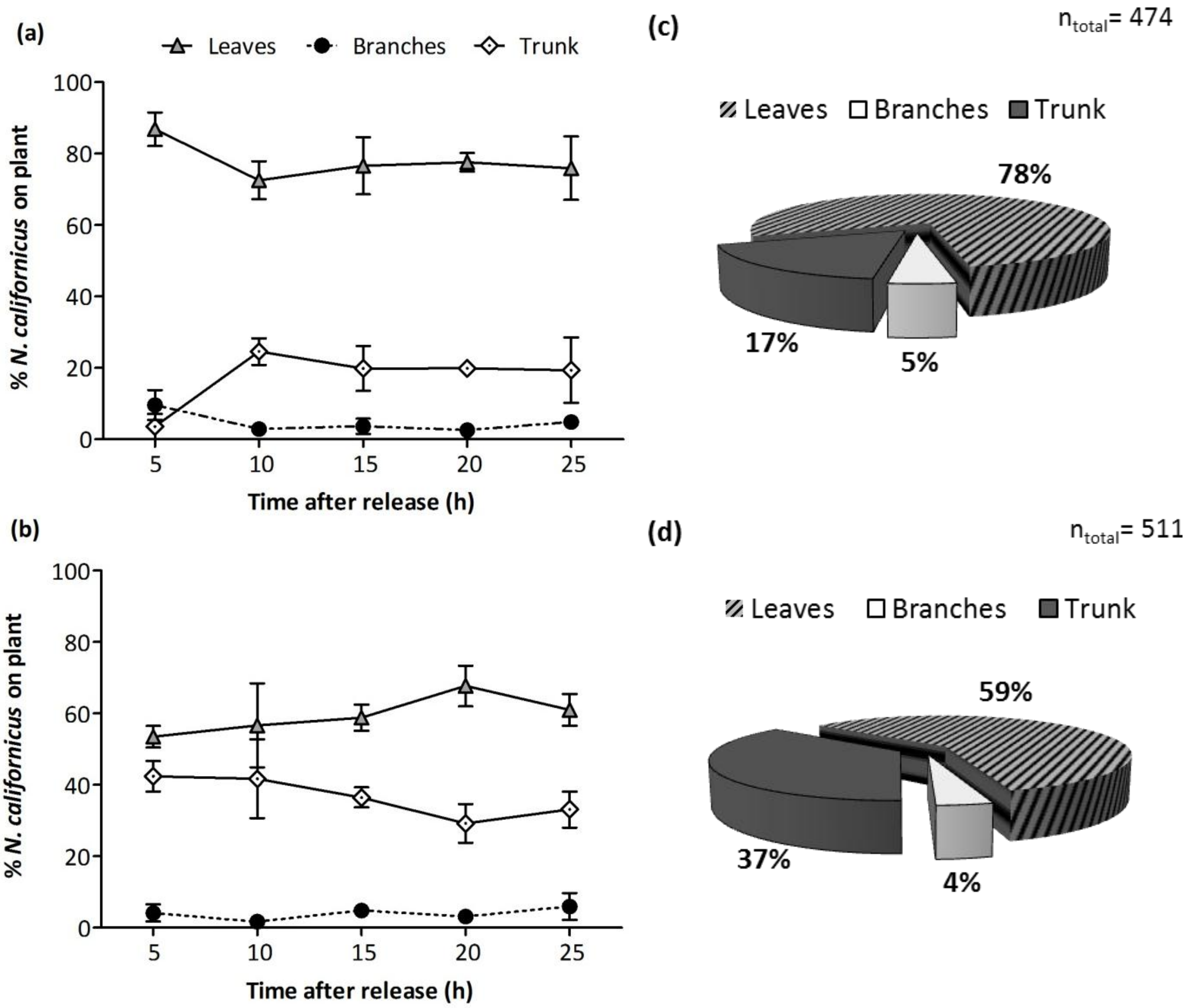

Fig. 5.2. Population dynamics (mean and SE, in %; a and b) and distribution (%) (c and d) of *N. californicus* in the three different substrates in both assays (assay 1: a, c; assay 2, b, d) on clementine plants.

5.4.3. Prey detection

Positive prey detection in *P. persimilis* and *N. californicus* was similar in both assays, both for specimens collected on leaves and on trunk + band and along the sampling time in all cases (Fisher's exact test, *P* > 0.05). On leaves, on average, positive prey detections for *P. persimilis* were around 97% whereas they were around 90% for *N. californicus* (Figure 5.3a). On trunk + band, positive prey detection was higher for *P. persimilis* (56%) than for *N. californicus* (13%) (Figure 5.3b).

Despite similar positive prey detection in both assays, a different number of phytoseiids was recovered in each one, especially for *N. californicus*. In the first assay, 10 *N. californicus* were found on trunk+band, whereas in the second one, 65 specimens were recovered from the same stratum. For *P. persimilis*, a similar number of specimens was found in each assay (11 and 10 individuals in assays 1 and 2, respectively). When positive prey detection was compared between substrates for each species and assay, significant differences were found (Fisher's exact test; *P* < 0.001 for both species).

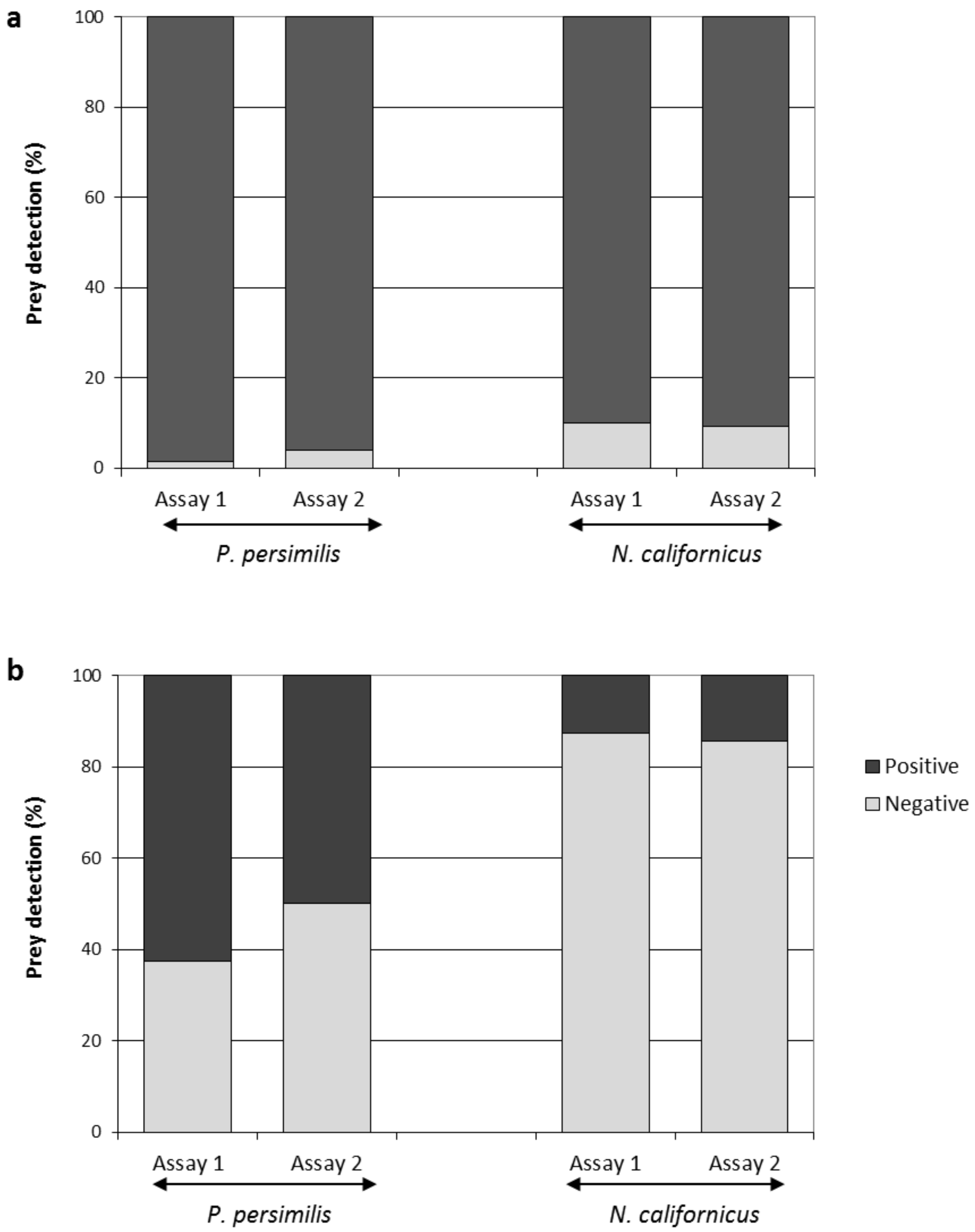

Fig. 5.3. Percentage of prey detections in leaves (a) and trunk plus band (b) on clementine plants in assays 1 and 2 for *P. persimilis* and *N. californicus*.

5.5. Discussion

Ours results show that the spatial distribution of *P. persimilis* and *N. californicus* along the sampling period (25 hours) was similar under different prey densities (assays 1 and 2), but differed between them, probably due to their different life-style (McMurtry & Croft 1997). While *P. persimilis* showed an uneven vertical distribution, independent of prey density, being more abundant in leaves than on branches and trunk, *N. californicus* distribution on plant substrates slightly changed depending on prey density. Higher prey densities (assay 1) made *N. californicus* more abundant on leaves than on trunk, whereas at lower prey density (assay 2), the percentage of this phytoseiid on trunk increased at the expenses of leaves.

Previous studies (Eveleigh & Chant 1982; Walzer et al. 2009; Alatawi et al. 2011) have reported that phytoseiids go in search of their prey, thus their effectiveness may be determined by the dispersal pattern of the prey, feeding habits, and the ability of the predator to find them and to reduce or eliminate their populations (Onzo et al. 2003; Kilpatrick & Ives 2003). In citrus, *T. urticae* is mainly found on leaves, where it lives grouped in colonies protected by the spiderweb (Martínez-Ferrer et al. 2006). In fact, we found the highest densities of *T. urticae* on this substrate whereas densities on branches and trunk were almost nil. Thus, the preference of both phytoseiids for occupying the leaves is probably linked to the presence of *T. urticae* in this substrate. Furthermore, field surveys on Spanish clementines also reported that *P. persimilis* and *N. californicus*, together with *E. stipulatus*, where the most abundant species associated with *T. urticae* colonies on the leaves (Abad-Moyano et al. 2009a).

In our study, *P. persimilis* was significantly more abundant on leaves, even at low prey density. Such a response was expected because it is considered a specialist predator, more voracious than others phytoseiid species, and highly dependent on aggregated prey mite species such as *T. urticae* (Zhang et al. 1992; McMurtry & Croft 1997; Pratt 1998; Shimoda & Takabayashi 2001; Walzer et al. 2009; Alatawi et al. 2011; Gontijo et al. 2012). Therefore, its survival directly depends on its prey (Gómez-Moya & Ferragut 2009). *Neoseiulus californicus* was also more abundant on leaves than on the trunk. However, it showed a higher tendency to move to the trunk and this behavior was enhanced at the lower densities (assay 2), where the numbers of *T. urticae* were minimal or nil, perhaps searching for alternative food or spatial refuge. This result supports other empirical evidences in which this predator shows dispersal characteristics more alike to those of a generalist predator. These features include a higher ability to move within the plant and to detect *T. urticae* infested-leaves with high or low density, despite showing a lower dependence on finding *T. urticae* (McMurtry & Croft 1997; Pratt 1998; Greco et al. 1999; Walzer et al. 2009).

Onzo et al (2003) described a spatiotemporal dynamics for *Typhlodromalus aripo* De Leon (Acari: Phytoseiidae). This mite took refuge on the apex of cassava plants during day-time and at night moved down to young leaves searching for the cassava green mite *Mononychellus tanajoa* (Bondar)(Acari: Tetranychidae). This migration was more frequent when prey density increased and this phytophagous mite feeds on young leaves. Although we have not recorded the individual movements of each phytoseiid among the substrates, we can generalize that neither *P. persimilis* nor *N. californicus* moved to search the prey or maybe five hours interval was too long to detect this movement. Both species, on both assays, remained mainly on leaves, where the prey was abundant. However, these predatory mites showed a different tendency to disperse (disappear) along the experiment. Whereas *N. californicus* numbers remained almost stable along the sampling time, *P. persimilis* numbers diminished as time passed, probably due to the exhaustion of the prey resource, and this tendency of disappearance was 2.7-fold greater than in *N. californicus*. For *P. persimilis* this dispersal behavior was observed for both prey densities and at a similar rate. This result is in agreement with the higher interplant dispersal rate described for *P. persimilis* (Skirvin & Fenlon 2003; Walzer et al. 2009; Alatawi et al. 2011).

Despite differences described in prey density at the beginning of the assay, positive prey detection was the same for both assays and no differences were observed along time for *P. persimilis* and *N. californicus* on the leaves. However, if we take into account the detectability half-lives, that is, the time elapsed until 50% of the consumed prey remains detectable in the gut of a predator (3 hours in *P. persimilis* and 13 hours in *N. californicus*) (Pina et al. 2011), the sampling time (5 hours intervals) and the percentage of positive prey detections, we can deduce that *P. persimilis* ate more frequently than *N. californicus*. This inferred superior rate of predation of *P. persimilis* when compared with *N. californicus* coincides with results previously reported by other authors (Friese & Gilstrap 1982; Escudero & Ferragut 1996). Furthermore, this result is reinforced by the results obtained for specimens collected on trunk + band, where more positive prey detections were found in *P.*

persimilis when compared with *N. californicus* even though fewer specimens of the former were recovered. Differences in positive prey detection between species on this substrate suggest that, apart from having a slightly different feeding behavior, they could be using this substrate in a different way. *Phytoseiulus persimilis* could have simply got stuck on the band when foraging, whereas *N. californicus* could have used it as a refuge, especially at low prey densities, because most individuals found there had not preyed on *T. urticae* at all (Figure 5.3b).

Understanding the distribution pattern of *P. persimilis* and *N. californicus*, their role as predators, and their establishment in the crop, can help make pest control through augmentative releases more effective. Currently, the only experimental strategy under development in augmentative biological control is based on hot spot releases of 100-500 *P. persimilis* per tree when economic threshold (20% of occupied leaves) is exceeded (Jacas & Urbaneja 2010). As a result of this study, we now know that *P. persimilis* preys actively on the leaves but tends to quickly disappear, probably due to prey exhaustion, avoiding the refuge site offered by the trunk. However, releases of *N. californicus*, even at low prey densities, could be also considered because they could continue preying without leaving the tree. In this case, the use of the trunk as a refuge explains the difficulty to detect it after release and the complexity to develop effective monitoring systems for these predatory mites in citrus. Whatever the case, we recommend the predatory mite releases to be carried out on prey patches in the leaves in order to facilitate their encounter with the prey, promoting an effective control.

CAPÍTULO 6

CONCLUSIONES

1. El neonicotinoide imidacloprid combinado con sueltas inoculativas de *Phytoseiulus persimilis* y *Neoseiulus californicus* es una alternativa factible al uso exclusivo del control químico dentro de la gestión integrada de plagas en viveros cítricos.

1.1. En ensayos de semi-campo, se comprobó que imidacloprid aplicado vía riego alteró ligeramente los parámetros demográficos de *N. californicus*. Sin embargo este fenómeno no fue observado en el caso de *P. persimilis*. Ello indica que el uso combinado de imidacloprid con sueltas de *P. persimilis*, preferiblemente frente a *N. californicus*, en condiciones de campo podría ser factible.

1.2. La aplicación de imidacloprid combinada con las sueltas inoculativas de *P. persimilis* en plantas de vivero redujo exitosamente las poblaciones del minador de las hojas de los cítricos *Phyllocnistis citrella*, de los pulgones *Aphis gossypii* y *A. spiraecola* y de la araña roja *Tetranychus urticae* en plantas jóvenes de clementino.

2. Basándonos en la toxicidad de los plaguicidas recomendados en cítricos sobre los ácaros depredadores más relevantes en este cultivo, *E. stipulatus* ha resultado ser la especie más tolerante, seguido de *N. californicus* y *P. persimilis* para los que la magnitud de los efectos secundarios de los plaguicidas estudiados fue muy parecida. Por ello, *E. stipulatus* no se considera como la especie idónea para servir como indicadora de los efectos secundarios de los plaguicidas en cítricos.

2.1. Productos como etoxazol, aceite mineral y spirotetramat fueron selectivos para las tres especies de fitoseidos estudiadas. Por ello, estos insecticidas son adecuados para su inclusión en programas de gestión integrada de plagas en los que *E. stipulatus*, *N. californicus* y *P. persimilis* sean relevantes como enemigos naturales.

2.2 *Phytoseiulus persimilis* demostró ser la especie más sensible de entre las estudiadas. Debido a su relevancia y eficacia en el control de *T. urticae*, este fitoseido debería ser utilizado como indicador de los efectos de los plaguicidas sobre los ácaros depredadores que aparecen en los huertos de cítricos en detrimento de *E. stipulatus*, que es la especie más utilizada hasta la fecha.

3. La calidad del polen afecta a la eficacia de los depredadores *N. californicus* y *E. stipulatus* en la regulación de las poblaciones de *T. urticae* en cítricos en condiciones de semi-campo. Este efecto se atribuye a los diferentes hábitos alimenticios de *N. californicus* (preferentemente entomófago) y *E. stipulatus* (omnívoro preferentemente palinófago) y podría tener importantes implicaciones en la regulación de las poblaciones de *T. urticae* en condiciones de campo.

3.1. La adición de polen, tanto de alta (*C. edulis*) como de baja (*F. arundinacea*) calidad, no supuso en nuestros ensayos, un mejor control de *T. urticae*. Sin embargo, el polen de alta calidad provocó explosiones poblacionales de *E. stipulatus*. Puesto que en clementino, *E. stipulatus* ejerce efectos letales tanto directos como indirectos sobre otras especies de fitoseido más eficientes en el control de *T. urticae*, como son *P. persimilis* y *N. californicus*, la sustitución de cubiertas multiflorales por cubiertas monoespecíficas de *F. arundinacea* podría ser un método de control biológico por conservación de *T. urticae*.

4. Las pautas de distribución espacial de los fitoseidos *P. persimilis* y *N. californicu*s, son ligeramente distintas, aunque ambas especies se encuentran mayoritariamente en hoja. *Phytoseiulus persimilis* fue consistentemente la especie más eficaz en la depredación de araña, tanto a densidades altas, como bajas de esta presa. Por todo ello, *P. persimilis*

aparece como la especie más adecuada para el control de *T. urticae* en clementino con sueltas dirigidas a los focos. Las hojas, especialmente aquellas con presencia de colonias de araña, son el sustrato donde debería centrase el muestreo de estos fitoseidos.

A modo de conclusión general, estos trabajos han proporcionado información valiosa con importantes implicaciones para la mejora de los programas de gestión de la araña roja *T. urticae* y su control biológico en los cítricos, que constituía el objetivo global de esta tesis.

REFERENCIAS BIBLIOGRÁFICAS

Abad-Moyano, R., Aguilar-Fenollosa, E. and Pascual-Ruiz, S. (2008) Control biológico de ácaros. *In* Jacas, J.A., and Urbaneja, A. (ed.) Control biológico de plagas agrícolas. M.V. Phytoma España, S.L., Valencia, España. p. 151-164.

Abad-Moyano, R., Pina, T., Dembilio, O., Ferragut, F. and Urbaneja, A. (2009a) Survey of natural enemies of spider mites (Acari: Tetranychidae) in citrus orchards in eastern Spain. Exp Appl Acarol 47:49-61.

Abad-Moyano, R., Pina, T., Ferragut, F. and Urbaneja, A. (2009b) Comparative life-history traits of three phytoseiid mites associated with *Tetranychus urticae* (Acari: Tetranychidae) colonies in clementine orchards in eastern Spain: implications for biological control. Exp Appl Acarol 47:121-132.

Abad-Moyano, R., Pina, T., Pérez-Panadés, J., Carbonell, E. and Urbaneja, A. (2010a) Efficacy of *Neoseiulus californicus* and *Phytoseiulus persimilis* in suppression of *Tetranychus urticae* in clementine nurseries. Exp Appl Acarol 50:317-328.

Abad-Moyano, R., Urbaneja, A., Hoffmann, D. and Schausberger, P. (2010b) Effects of *Euseius stipulatus* on establishment and efficacy in spider mite suppression of *Neoseiulus californicus* and *Phytoseiulus persimilis* in clementine. Exp Appl Acarol 50:329-341.

Abad-Moyano, R., Urbaneja, A. and Schausberger, P. (2010c) Intraguild interactions between *Euseius stipulatus* and the candidate biocontrol agents of *Tetranychus urticae* in Spanish clementine orchards: *Phytoseiulus persimilis* and *Neoseiulus californicus*. Exp Appl Acarol 50:23-34.

Abbott, W.S. (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265-267.

Aguilar-Fenollosa, E., Ibanez-Gual, M.V., Pascual-Ruiz, S., Hurtado, M. and Jacas, J.A. (2011a) Effect of ground-cover management on spider mites and their phytoseiid natural enemies in clementine mandarin orchards (I): Bottom-up regulation mechanisms. Biol Control 59:158-170.

Aguilar-Fenollosa, E., Ibanez-Gual, M.V., Pascual-Ruiz, S., Hurtado, M. and Jacas, J.A. (2011b) Effect of ground-cover management on spider mites and their phytoseiid natural enemies in clementine mandarin orchards (II): Top-down regulation mechanisms. Biol Control 59:171-179.

Aguilar-Fenollosa, E., Pascual-Ruiz, S., Hurtado, M.A. and Jacas, J.A. (2011c) Efficacy and economics of ground cover management as a conservation biological control strategy against *Tetranychus urticae* in clementine mandarin orchards. Crop Prot 30:1328-1333.

Aguilar-Fenollosa, E., Pina, T., Gómez-Martínez, M., Hurtado, M.A. and Jacas, J.A. (2012) Does host adaptation of *Tetranychus urticae* populations in clementine orchards with a *Festuca arundinacea* cover contribute to a better natural regulation of this pest mite? Entomol Exp Appl 144:181-190.

Ahmadi, M., Fathipur, Y. and Kamali, K. (2005) Population growth parameters of *Tetranychus urticae* (Acari: Tetranychidae) on different bean varieties. J Entomol Soc Iran 26:1-10.

Akaike, H. (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19:716-723.

Ako, M., Poehling, H.M., Borgemeisterl, C. and Nauen, R. (2006) Effect of imidacloprid on the reproduction of acaricide-resistant and susceptible strains of *Tetranychus urticae* Koch (Acari: Tetranychidae). Pest Manag Sci 62:419-424.

Alatawi, F., Nechols, J.R. and Margolies, D.C. (2011) Spatial distribution of predators and prey affect biological control of twospotted spider mites by *Phytoseiulus persimilis* in greenhouses. Biol Control 56:36-42.

Alston, D.G. (1994) Effect of apple orchard floor vegetation on density and dispersal of phytophagous and predaceous mites in Utah. Agr Ecosyst Environ 50:73-84.

Alvis, L. 2003. Identificación y abundancia de artrópodos depredadores en los cultivos de cítricos Valencianos. Tesis Doctoral, Universidad Politécnica de Valencia.

Anh, K., Lee S.Y., Lee, K., Lee, Y. and Kim, G. (2004) Selective toxicity of pesticides to the predatory mite, *Phytoseiulus persimilis* and control effects of the two-spotted spider mite, *Tetranychus urticae* by predatory mite and pesticide mixture on rose. Kor J Appl Entomol 43:71-79.

Ansaloni, T., Pascual-Ruiz, S., Hurtado, M.A. and Jacas, J.A. (2008) Can summer and fall vegetative growth regulate the incidence of *Tetranychus urticae* Koch on clementine fruit? Crop Prot 27:459-464.

Apostol, T.M. (1974) Mathematical Analysis. Addison-Wesley, Reading, Massachusetts.

Arbona, V., Flors, V., Jacas, J.A., Garcia, P. and Gómez-Cadenas, A. (2003) Enzymatic and non-enzymatic antioxidant responess of *Carrizo citrange*, a salt-sensitive citrus rootstock, to different levels of salinity. Plant Cell Physiol 44:388-394.

Argolo, P.S., Banyuls, N., Santiago, S., Mollá, O. and Urbaneja, A. (2012) Compatibility of *Phytoseiulus persimilis* and *Neoseiulus californicus* (Acari: Phytoseiidae) with imidacloprid to manage clementine nursery pests. Crop Prot , *in press*.

Aucejo, S.F.M., Gimeno, E., Gómez-Cadenas, A., Monfort, R., Obiol, F., Prades, E., Ramis, M., Ripollés, J.L., Tirado, V., Zaragoza, L., Jacas, J.A. and Martínez-Ferrer, M.T. (2003) Management of *Tetranychus urticae* in citrus in Spain: acarofauna associated to weeds. IOBC/wprs Bull 26:213-220.

Aucejo-Romero, S. 2005. Manejo Integrado de *Tetranychus urticae* Koch (Acari: Tetranychidae) en clementinos: agregación, dinámica e influencia del estado nutricional de la planta huésped. Tesis Doctoral, Universidad Politécnica de Valencia.

Aucejo-Romero, S., Foó, M., Ramis, M., Troncho, P., Gómez-Cadenas, A. and Jacas, J.A. (2003) Evaluación de la eficacia de algunos acaricidas contra la araña roja, *Tetranychus urticae* Koch (Acari: Tetranychidae), en clementino. Bol San Veg Plagas 29:453-459.

Aucejo-Romero, S., Gómez-Cadenas, A. and Jacas-Miret, J.A. (2004) Effects of NaCl-stressed citrus plants on life-history parameters of *Tetranychus urticae* (Acari: Tetranychidae). Exp Appl Acarol 33:55-68.

Auger, P., Tixier, M.S., Kreiter, S. and Fauvel, G. (1999) Factors affecting ambulatory dispersal in the predaceous mite *Neoseiulus californicus* (Acari: Phytoseiidae). Exp Appl Acarol 23:235-250.

Baal, E. v., Houten, Y. V. v., Hoogerbrugge, H. and Bolckmans, K. (2007) Side effects on thrips of the spider mite predator *Neoseiulus californicus*. Proc Neth Entomol Soc Meet 18:37-42.

Badii, M.H., Landeros, J. and Cerna, E. (2011) Regulación poblacional de ácaros plaga de impacto agrícola. Daena Int J Good Conscienc 5:270-302.

Badii, M.H. and McMurtry, J.A. (1984) Life-History of and life table parameters for *Phytoseiulus longipes* with comparative studies on *Phytoseiulus persimilis* and *Typhlodromus occidentalis* (Acari: Phytoseiidae). Acarol 25:111-123.

Barbosa, P. (1998) Conservation Biological Control. Barbosa, P. (ed.) Academic Press, San Diego, CA (USA).

Bellows, T.S. and Morse, J.G. (1988) Residual toxicity following dilute or low-volume applications of insecticides used for control of California red scale (Homoptera: Diaspididae) to four beneficial species in a citrus agroecosystem. J Econ Entomol 81:892-898.

Bellows, T.S., Morse, J.G. and Gaston, L.K. (1992) Residual toxicity of pesticides used for control of lepidopteran insects in citrus to the predaceous mite *Euseius stipulatus* Athias-Henriot (Acarina, Phytoseiidae). J Appl Entomol 113:493-501.

Biobest (2012) Biobest Sustanible Crop Management http://www.biobest.be/ Accessed: 4-5-2012

Blümel, S., Matthews, G.A., Grinstein, A. and Elad, Y. (2002) Pesticides in IPM: Selectivity, side-effects, application and resistance problems integrated pest and disease management in greenhouse crops. *In* Albajes, R., Lodovica Gullino, M., Lenteren, J. C., and Elad, Y. (ed.) Springer Netherlands, p. 150-167.

Bostanian, N.J. and Akalach, M. (2006) The effect of indoxacarb and five other insecticides on *Phytoseiulus persimilis* (Acari: Phytoseiidae), *Amblyseius fallacis* (Acari: Phytoseiidae) and nymphs of *Orius insidiosus* (Hemiptera: Anthocoridae). Pest Manag Sci 62:334-339.

Bostanian, N.J., Hardman, J.M., Thistlewood, H.A. and Racette, G. (2010) Effects of six selected orchard insecticides on *Neoseiulus fallacis* (Acari: Phytoseiidae) in the laboratory. Pest Manag Sci 66:1263-1267.

Bostanian, N.J., Thistlewood, H.A., Hardman, J.M., Laurin, M.C. and Racette, G.T. (2009) Effect of seven new orchard pesticides on *Galendromus occidentalis* in laboratory studies. Pest Manag Sci 65:635-639.

Bouras, S.L. and Papadoulis, G.T. (2005) Influence of selected fruit tree pollen on life history of *Euseius stipulatus* (Acari: Phytoseiidae). Exp Appl Acarol 36:1-14.

Bruessow, F., Asins, M.J., Jacas, J.A. and Urbaneja, A. (2010) Replacement of CTV-susceptible sour orange rootstock by CTV-tolerant ones may have triggered outbreaks of *Tetranychus urticae* in Spanish citrus. Agricult Ecosys Environ 137:93-98.

Carey, J.R. and Bradley, J.W. (1982) Developmental rates, vital schedules, sex-ratios, and life-tables for *Tetranychus urticae*, *Tetranychus turkestani* and *Tetranychus pacificus* (Acarina: Tetranychidae) on Cotton. Acarol 23:333-345.

Castagnoli, M., Liguori, M., Simoni, S. and Duso, C. (2005) Toxicity of some insecticides to *Tetranychus urticae*, *Neoseiulus californicus* and *Tydeus californicus*. BioControl 50:611-622.

Castagnoli, M. and Simoni, S. (1999) Effect of long-term feeding history on functional and numerical response of *Neoseiulus californicus* (Acari : Phytoseiidae). Exp Appl Acarol 23:217-234.

Castagnoli, M. and Simoni, S. (2003) *Neoseiulus californicus* (McGregor) (Acari Phytoseiidae): survey of biological and behavioural traits of a versatile predator. Redia 86:153-164.

Castagnoli, M. and Simoni, S. (1991) Influenza Della Temperature Sull'incremento Delle Popolazioni di *Amblyseius californicus* (McGregor) (Acari: Phytoseiidae). Redia 74:621-640.

Castle, S.J. and Prabhaker, N. (2011) Field evaluation of two systemic neonicotinoid insecticides against pink hibiscus mealybug (*Maconellicoccus hirsutus* (Green)) on mulberry trees. J Pest Sci 84:363-371.

Chong, J.H., Hodges, G.S. and Samuel-Foo, M. (2009) First record and management of the armored scale, *Melanaspis deklei* Dietz & Davidson (Hemiptera: Diaspididae), in South Carolina. J Agr Urban Entomol 26:63-75.

Clark, T.L. and Messina, F.J. (1998) Foraging behavior of lacewing larvae (Neuroptera: Chrysopidae) on plants with divergent architectures. J Insect Behav 11:303-317.

Costa-Comelles, J., Alonso, A., Rodriguez, J.M. and Garcia-Marí, F. (1994) El pulgón *Aphis gossypii* Glover: eficacia de algunos plaguicidas en cítricos y su acción sobre el fitoseído *Euseius stipulatus*. Levante Agrícola 328:201-213.

Costa-Comelles, J., Ferragut, F., García-Marí, F., Laborda, R. and Marzal, C. (1986) Abundancia y dinámica poblacional de las especies de ácaros que viven en los manzanos de Lérida. Agricola Vergel 51:176-191.

Croft, B.A. (1990) Management of pesticide resistance in arthropod pests. *In* Green, M. B., Moberg, W. K., and Lebaron, H. (ed.) Managing resistance to agrochemicals: fundamental and practical approaches to combating resistance. Ame Chem Soc, Washington, DC. p. 149-168.

Croft, B.A., Blackwood, J.S. and McMurtry, J.A. (2004) Classifying life-style types of phytoseiid mites: diagnostic traits. Exp Appl Acarol 33:247-260.

Croft, B.A., Monetti, L.N. and Pratt, P.D. (1998) Comparative life histories and predation types: Are *Neoseiulus californicus* and *N. fallacis* (Acari: Phytoseiidae) similar type II selective predators of spider mites? Environ Entomol 27:531-538.

Daane, K.M., Bentley, W.J., Millar, J.G., Walton, V.M., Cooper, M.L., Biscay, P. and Yokota, G.Y. (2008) Integrated management of mealybugs in California vineyards. p. 235-252.

De Mendoza, A.H., Arouni, R., Belliure, B., Carbonell, E.A. and Pérez-Panadés, J. (2006) Intervention thresholds for *Aphis spiraecola* (Hemiptera: Aphididae) on Citrus clementina. J Econ Entomol 99:1273-1279.

Dupont, L.M. (1979) On gene flow between *Tetranychus urticae* Koch, 1836 and *Tetranychus cinnabarinus* (Boisduval) Boudreaux, 1956 (Acari: Tetranychidae): Synonomy between the two species. Entomol Exp Appl 25:297-303.

Duso, C., Malagnini, V., Pozzebon, A., Castagnoli, M., Liguori, M. and Simoni, S. (2008) Comparative toxicity of botanical and reduced-risk insecticides to Mediterranean populations of *Tetranychus urticae* and *Phytoseiulus persimilis* (Acari Tetranychidae, Phytoseiidae). Biol Control 47:16-21.

Duso, C., Malagnini, V., Paganelli, A., Aldegheri, L., Bottini, M. and Otto, S. (2004) Pollen availability and abundance of predatory phytoseiid mites on natural and secondary hedgerows. BioControl 49:397-415.

Elbert, A., Becker, B., Hartwig, J. and Erdelen, C. (1991) Imidacloprid - a new systemic insecticide. Pflanzensch -Nachr Bayer 44:113-136.

Escudero, A. and Ferragut, F. (1996) Comportamiento de *Phytoseiulus persimilis* Athias-Henriot y *Neoseiulus californicus* (McGregor) (Acari:Phytoseiidae) ante diferentes densidades de presa. Bol San Veg Plagas 22:115-124.

Escudero, L.A. and Ferragut, F. (2005) Life-history of predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis* (Acari : Phytoseiidae) on four spider mite species as prey, with special reference to *Tetranychus evansi* (Acari : Tetranychidae). Bio Control 32:378-384.

EU (2009) Directiva 2009/128/EC del Parlamento Europeo de 21 de octubre de 2009 por la que se establece el marco de acción comunitario para alcanzar un uso sostenible de plaguicidas. OJEU L 309:71-86.

Eveleigh, E.S. and Chant, D.A. (1982) The searching behaviour of two species of phytoseiid mites, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius degenerans* (Berlese), in relation to the density and distribution of prey in an homogeneous area (Acarina: Phytoseiidae). Can J Zool 60:648-658.

FAOSTAT (2010) Food and Agriculture Organization of the United Nations http://faostat.fao.org/site/339/default.aspx Accessed: 30-5-2012

Ferragut, F., Costa-Comelles, J., García-Marí, F., Laborda, R., Roca, D. and Marzal, C. (1988) Dinámica poblacional del fitoseido *Euseius stipulatus* (Athis-Henriot) y su presa *Panonychus citri* (McGregor) (Acari: Phytoseidae, Tetranychidae), en los cítricos españoles. Bol San Veg Plagas 14:45-54.

Ferragut, F., Garcia-Marí, F., Costa-Comelles, J. and Laborda, R. (1987) Influence of food and temperature on development and oviposition of *Euseius stipulatus* and *Typhlodromus phialatus* (Acari: Phytoseiidae). Exp Appl Acarol 3:317-329.

Ferragut, F., Laborda, R., Costa-Comelles, J. and García-Marí, F. (1992) Feeding-behavior of *Euseius stipulatus* and *Typhlodromus phialatus* on the citrus red mite *Panonychus citri* [Acari, Phytoseiidae-Tetranychidae]. Entomophaga 37:537-543.

Fitzgerald, J., Xu, X., Pepper, N., Easterbrook, M. and Solomon, M. (2008) The spatial and temporal distribution of predatory and phytophagous mites in field-grown strawberry in the UK. Exp Appl Acarol 44:293-306.

Flechtmann, C.H.W. and McMurtry, J.A. (1992a) Studies on how phytoseiid mites feed on spider mites and pollen. Int J Acarol 18:157-162.

Flechtmann, C.H.W. and McMurtry, J.A. (1992b) Studies of cheliceral and deutosternal morphology of some phytoseiidae (Acari: Mesostigmata) by scanning electron microscopy. Int J Acarol 18:163-169.

Flexner, J.L., Westigard, P.H., Gonzalves, P. and Hilton, R. (1991) The effect of ground groundcover and herbicide treatment on twospotted spider mite density and dispersal in southern Oregon pear orchards. Exp Appl Acarol 60:111-123.

Friese, D.D. and Gilstrap, F.E. (1982) Influence of prey availability on reproduction and prey consumption of *Phytoseiulus persimilis*, *Amblyseius californicus* and *Metaseiulus occidentalis* (Acarina: Phytoseiidae). Int J Acarol 8:85-89.

García-Marí, F. and Ferragut, F. (2002) Los Ácaros. *In* García-Marí, F., and Ferragut, F. (ed.) Plagas Agrícolas. Phytoma-España S.L., Valencia. p. 19-52.

García-Marí, F., Ferragut, F., Costa-Comelles, J., Laborda, R., Santabala, E., Ferragut, F., Marzal, C., Colomer, P. and Costa, J. (1983) El ácaro rojo *Panonychus citri* (McGregor): incidencia en la problemática fitosanitaria de nuestros agrios. Bol Serv Plagas 9:191-218.

García-Marí, F., Ferragut, F., Marzal, C., Costa-Comelles, J. and Laborda, R. (1986) Ácaros que viven en las hojas de los cítricos españoles. Inv Agr Prod Prot Veg 1:219-250.

García-Marí, F., Llorens, J.M., Costa-Comelles, J. and Ferragut, F. (1991) Ácaros de las plantas cultivadas y su control biológico. Ediciones Pisa, 175 Alicante, Spain.

García-Marí, F. and Rivero, J.M.D. (1984) The citrus red mite, *Panonychus citri*, a new pest of citrus in Spain. EPPO Bull 14:129-134.

Garrido, A. and Ventura, J.J. (1993) Plagas de los cítricos. Bases para el manejo integrado. Ministerio de Agricultura, Pesca y Alimentación (ed.) Dirección general de Sanidad de la Producción Agraria, Madrid, Spain.

Gómez de Barreda, D. (1994) Sistemas de manejo del suelo en citricultura. Generalitat Valenciana, Silla, Spain.

Gómez-Moya, C.A. and Ferragut, F. (2009) Distribución en la planta y eficacia de *Neoseiulus californicus* y *Phytoseiulus persimilis* (Acari: Phytoseiidae) en el control de las arañas rojas de cultivos hortícolas en condiciones de semi-campo. Bol San Veg Plagas 35:377-390.

Gontijo, L.M., Margolies, D.C., Nechols, J.R. and Cloyd, R.A. (2010) Plant architecture, prey distribution and predator release strategy interact to affect foraging efficiency of the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae) on cucumber. Biol Control 53:136-141.

Gontijo, L.M., Nechols, J.R., Margolies, D.C. and Cloyd, R.A. (2012) Plant architecture and prey distribution influence foraging behavior of the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae). Exp Appl Acarol 56:23-32.

González-Fernández, J.J., de la Peña, F., Hormaza, J.I., Boyero, J.R., Vela, J.M., Wong, E., Trigo, M.M. and Montserrat, M. (2009) Alternative food improves the combined effect of an omnivore and a predator on biological pest control. A case study in avocado orchards. Bull Entomol Res 99:433-444.

Gotoh, T., Yamaguchi, K. and Mori, K. (2004) Effect of temperature on life history of the predatory mite *Amblyseius* (*Neoseiulus*) *californicus* (Acari: Phytoseiidae). Exp Appl Acarol 32:15-30.

Grafton-Cardwell, E.E., Lee, J.E., Robillard, S.M. and Gorden, J.M. (2008) Role of imidacloprid in Integrated Pest Management of California citrus. J Econ Entomol 101:451-460.

Grafton-Cardwell, E.E., Ouyang, Y.L. and Striggow, R.A. (1997) Predaceous mites (Acari: Phytoseiidae) for control of spider mites (Acari: Tetranychidae) in nursery citrus. Environ Entomol 26:121-130.

Grafton-Cardwell, E.E., Ouyang, Y. and Bugg, R.L. (1999) Leguminous Cover Crops to Enhance Population Development of *Euseius tularensis* (Acari: Phytoseiidae) in Citrus. Biol Control 16:73-80.

Grbic, M., Khila, A., Lee, K.Z., Bjelica, A., Grbic, V., Whistlecraft, J., Verdon, L., Navajas, M. and Nagy, L. (2007) Mity model: *Tetranychus urticae*, a candidate for chelicerate model organism. BioEssays 29:489-496.

Greco, N.M., Liljesthrom, G.G. and Sanchez, N.E. (1999) Spatial distribution and coincidence of *Neoseiulus californicus* and *Tetranychus urticae* (Acari : Phytoseiidae, Tetranychidae) on strawberry. Exp Appl Acarol 23:567-580.

GVA (2012) Boletín de Avisos Abril Núm 5. Cítrics http://www.agricultura.gva.es/web/c/document_library/get_file?uuid=25b68967-dbf9-4ab8-9fc2-cc83f70af5e3&groupId=16 Accessed: 4-5-2012

Hamamura, T., Shinkaji, S. and Ashihara, W. (1976) The relationship between temperature and developmental period and oviposition of *Phytoseiulus persimilis* (Acarina: Phytoseiidae). Bull Fruit Tree Res Stn E 1:117-125.

Hardman, J.M., Jensen, K.I.N., Franklin, J.L. and Moreau, D.L. (2005) Effect of dispersal, predators (Acari: Phytoseiidae), weather, and ground cover treatments on populations of *T. urticae* (Acari: Tetranychidae) in Apple Orchards. J Hortic Entomol 98:862-874.

Hare, J.D. (1994) Sampling arthropod pests in citrus. Pedigo, L.P. and Buntin, G.D. (ed.) CRC Press, 428 .

Hassan, E., Oomen, P.A., Overmeer, W.P.J., Plevoets, P., Reboulet, J.N., Rieckmann, W., Samsoe-Petersen, L., Shires, S.W., Stäubli, A., Stevenson, J., Tuset, J.J., Vanwetswinkel, G. and van Zon, A.Q. (1985) Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS Working Group "Pesticides and Beneficial Organisms". EPPO Bull 15:214-255.

Hassan, S.A. (1994) Activities of the IOBC/wprs working group Pesticides and Beneficial Organisms. IOBC/wprs Bull 17:1-5.

Hassan, S.A. (1977) Standardized techniques for testing side-effects of pesticides on beneficial arthropods in the laboratory. Z Pflanzenkrankh Pflanzensch 84:158-163.

Hassan, S.A., Bigler, F., Bogenschütz, H., Boller, E., Brun, J., Chiverton, P., Edwards, P., Mansour, F., Naton, E., Oomen, P.A., Overmeer, W.P.J., Polgar, L., Rieckmann, W., Samsoe-Petersen, L., Stäubli, A.A., Sterk, G., Tavares, K., Tuset, J.J., Viggiani, G. and Vivas, A.G. (1988) Results of the fourth joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". Z Angew Entomol 105:321-329.

Hassan, S.A. and Oomen, P.A. (1985) Testing the side effects of pesticides on beneficial organisms by OILBWorking Party. *In* Hussey, N. W., and Scopes, N. (ed.) Biological Pest Control – the Glasshouse Experience. Blandford Press, Poole, Dorset. p. 145-152.

Helle, W. and Sabelis, M.W. (1985b) World crop pests, vol. 1A. Spider mites: their biology, natural enemies and control. Elsevier, Amsterdam, The Netherlands.

Helle, W. and Sabelis, M.W. (1985a) World crop pests, vol. 1B. Spider mites: their biology, natural enemies and control. Elsevier, Amsterdam, The Netherlands.

Herbert, H.J. (1981) Biology, life-tables, and innate capacity for increase of the twospotted spider-mite, *Tetranychus urticae* (Acarina, Tetranychidae). Can Entomol 113:371-378.

Ichinose, K., Bang, D.V., Tuan, D.H. and Dien, L.Q. (2010) Effective use of neonicotinoids for protection of citrus seedlings from invasion by *Diaphorina citri* (Hemiptera: Psyllidae). J Econ Entomol 103:127-135.

IVEX (2012) Instituto Valenciano de la Exportación http://www.ivex.es/estudios/informacion_sectorial_cv.html

Jacas, J.A. and Garcia-Marí, F. (2001) Side-effects of pesticides on selected natural enemies occurring in citrus in Spain. IOBC/wprs Bull 24:103-112.

Jacas, J.A., Karamaouna, F., Vercher, R. and Zappalà, L. (2010) Citrus Pest Management in the Northern Mediterranean Basin (Spain, Italy and Greece). *In* Ciancio, A., and Mukerji, K. G. (ed.) Integrated Management of Arthropod Pests and Insect Borne Diseases. Springer Netherlands, Dordrecht, NL. p. 3-27.

Jacas, J.A. and Urbaneja, A. (2010) Biological Control in Citrus in Spain: from classical to conservation biological control. *In* Ciancio, A., and Mukerji, K. G. (ed.) Integrated Management of Plant Pests and Diseases. Springer Netherlands, Dordrecht, NL. p. 61-72.

Jacas, J.A. and Urbaneja, A. (2008) Control biológico de plagas agrícolas. Jacas, J.A. and Urbaneja, A. (ed.) M.V. Phytoma España, S.L., 496 Valencia, Spain.

James, D.G. (1997) Imidacloprid increases egg production in *Amblyseius victoriensis* (Acari: Phytoseiidae). Exp Appl Acarology 21:75-82.

James, D.G. (2002) Selectivity of the acaricide, bifenazate, and aphicide, pymetrozine, to spider mite predators in Washington hops. Int J Acarol 28:175-179.

James, D.G. (2003) Toxicity of imidacloprid to *Galendromus occidentalis*, *Neoseiulus fallacis* and *Amblyseius andersoni* (Acari: Phytoseiidae) from hops in Washington State, USA. Exp Appl Acarol 31:275-281.

James, D.G. and Price, T.S. (2002) Fecundity in twospotted spider mite (Acari: Tetranychidae) is increased by direct and systemic exposure to imidacloprid. J Econ Entomol 95:729-732.

James, D.G. and Vogele, B. (2001) The effect of imidacloprid on survival of some beneficial arthropods. Plant Prot Quart 16:58-62.

Janssen, A. and Sabelis, M.W. (1992) Phytoseiid life-histories, local predator-prey dynamics, and strategies for control of Tetranychid mites. Exp Appl Acarol 14:233-250.

Jonsson, M., Wratten, S.D., Landis, D.A. and Gurr, G.M. (2008) Recent advances in conservation biological control of arthropods by arthropods. Biol Control 45:172-175.

Juan-Blasco, M., San Andrés, V. , Martínez-Utrillas, M.A. , Argilés, R. , Pla, I. , Urbaneja, A. and Sabater-Muñoz, B. (2012) Alternatives to ginger root oil aromatherapy for improved mating performance of sterile *Ceratitis capitata* (Diptera: Tephritidae) males. J Appl Entom, *in press*.

Karamaouna, F., Pascual-Ruiz, S., Aguilar-Fenollosa, E., Verdú, M.J., Urbaneja, A. and Jacas, J.A. (2010) Changes in predation and parasitism of the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) populations in Spain following establishment of *Citrostichus phyllocnistoides* (Hymenoptera: Eulophidae). Biol Control 52:37-45.

Katayama, H., Masui, S., Tsuchiya, M., Tatara, A., Doi, M., Kaneko, S. and Saito, T. (2006) Density suppression of the citrus red mite *Panonychus citri* (Acari: Tetranychidae) due to the occurrence of *Neoseiulus californicus* (McGregor) (Acari : Phytoseiidae) on Satsuma mandarin. Appl Entomol Zool 41:679-684.

Kennett, C., Flaherty, D. and Hoffmann, R. (1979) Effect of wind-borne pollens on the population dynamics of *Amblyseius hibisci* [Acarina: Phytoseiidae]. BioControl 24:83-98.

Khalequzzaman, M., Mondal, M., Haque, M.F. and Karim, M.S. (2007) Predatory efficacy of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) on the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). J Bio-Sci 15:127-132.

Kilpatrick, A.M. and Ives, A.R. (2003) Species interactions can explain Taylor's power law for ecological time series. Nature 422:65-68.

Kim, D.S. and Lee, J.H. (2003) Oviposition model of overwintered adult *Tetranychus urticae* (Acari: Tetranychidae) and mite phenology on the ground cover in apple orchards. Entomol Exp Appl 31:191-208.

Kim, S.S., Seo, S.G., Park, J.D., Kim, S.G. and Kim, D.I. (2005) Effects of selected pesticides on the predatory mite, *Amblyseius cucumeris* (Acari : Phytoseiidae). J Entomol Sci 40:107-114.

Kim, S.S. and Yoo, S.S. (2002) Comparative toxicity of some acaricides to the predatory mite, *Phytoseiulus persimilis* and the twospotted spider mite, *Tetranychus urticae*. BioControl 47:563-573.

Koppert (2012) Koppert Biological Systems http://www.koppert.es/ Accessed: 4-3-2011

Lacasa, A. and Llorens, J.M. (1998) Trips y su control biológico (II). Alicante, España.

Landis, D.A., Wratten, S.D. and Gurr, G.M. (2000) Habitat management to conserve natural enemies of athropod pests in agriculture. Annu Rev Entomol 45:175-201.

Legrand, A. and Barbosa, P. (2003) Plant morphological complexity impacts foraging efficiency of adult *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). Environ Entomol 32:1219-1226.

Liang, W. and Huang, M. (1994) Influence of citrus orchard ground cover plants on arthropod communities in China: A review. Agr Ecosyst Environ 50:29-37.

Lilley, R. (1999) Vertical dispersal of the two-spotted spider mite *Tetranychus urticae*, and the predatory mite *Phytoseiulus persimilis* on dwarf hops. Agr Forest Entomol 1:111-117.

Liu, C., Wang, G., Wang, W. and Zhou, S. (2002) Spatial pattern of *Tetranychus urticae* population in apple tree garden. Chin J Appl Ecol 13:993-996.

Lundgren, J.G. (2009) Relationships of Natural Enemies and Non-Prey Foods. The Pollen feeders. Progress in Biological Control Vol. 7. Springer Netherlands, p. 87-116.

Macke, E., Magalhaes, S., Khan, H.D.T., Luciano, A., Frantz, A., Facon, B. and Olivieri, I. (2011) Sex allocation in haplodiploids is mediated by egg size: evidence in the spider mite *Tetranychus urticae* Koch. Proc Royal Soc Biol Sci 278:1054-1063.

MAGRAMA (2010) Anuario de Estadística Ministerio de Agricultura, Alimentación y Medio Ambiente http://www.magrama.gob.es/estadistica/pags/anuario/2011/AE_2011_Avance.pdf Accessed: 2-2-2012

MAGRAMA (2012) Ministerio de Medio Ambiente y Medio Rural y Marino. Registro de productos fitosanitarios http://www.magrama.es/es/agricultura/temas/medios-de-produccion/productos-fitosanitarios/registro/menu.asp Accessed: 4-3-2012

Mailloux, J., Le Bellec, F., Kreiter, S., Tixier, M.S. and Dubois, P. (2010) Influence of ground cover management on diversity and density of phytoseiid mites (Acari: Phytoseiidae) in Guadeloupean citrus orchards. Exp Appl Acarol 52:275-290.

Maoz, Y., Gal, S., Abrahams, Y., Gan-Mor, S., Coll, M., and Palevsky, E. (2009) Pollen provisioning enhances *Euseius scutalis* (Phytoseiidae) populations and improves control of *Olygonychus persea* (Tetranychidae), in: Mason

Maoz, Y., Gal, S., Argov, Y., Coll, M. and Palevsky, E. (2011) Biocontrol of persea mite, *Oligonychus perseae*, with an exotic spider mite predator and an indigenous pollen feeder. Biol Control 59:147-157.

Martínez-Ferrer, M.T. (2003) Biología y control del cotonet *Planococcus citri* (Homoptera: Pseudococcidae) en huertos cítricos. Tesis Doctoral, Universidad Politécnica de Valencia.

Martínez-Ferrer, M.T., Jacas, J.A. and Ripollés, J.L. (2004) La araña roja, *Tetranychus urticae*: métodos de muestreo y umbrales económicos de tratamiento en clementinos. Phytoma España 164:53-58.

Martínez-Ferrer, M.T., Jacas, J.A., Ripollés-Moles, J.L., and Aucejo-Romero, S. (2005) Sampling plans for *Tetranychus urticae* (Acari: Tetranychidae) for IPM decisions on Clementines in Spain IOBC/wprs Working Group "Integrated Control in Citrus Fruit Crops" Lisboa, Portugal.

Martínez-Ferrer, M.T., Jacas, J.A., Ripollés-Moles, J.L. and Aucejo-Romero, S. (2006) Approaches for sampling the twospotted spider mite (Acari: Tetranychidae) on Clementines in Spain. J Econ Entomol 99:1490-1499.

McMurtry, J.A. (1982) The use of phytoseiids for biological control: progress and future prospects. *In* Hoy, M. A. (ed.) Recent Advances in Knowledge of the Phytoseiidae. Univ Calif Div Agric Sci Publ, Berkeley. p. 23-48.

McMurtry, J.A. (1977) Some predaceous mites (Phytoseidae) on citrus in the Mediterranean region. Entomophaga 22:19-60.

McMurtry, J.A. (1992) Dynamics and potential impact of "generalist" phytoseiids in agroecosystems and possibilities for establishment of exotic species. Exp Appl Acarol 14:371-382.

McMurtry, J.A. and Croft, B.A. (1997) Life-styles of phytoseiid mites and their role in biological control. Annu Rev Entomol 42:291-321.

McMurtry, J.A. and Scriven, G.T. (1966a) The influence of pollen and prey density on the numbers of prey consumed by *Amblyseius hibisci* (Acarina: Phytoseiidae). Ann Entomol Soc Am 59:149-157.

McMurtry, J.A. and Scriven, G.T. (1966b) Studies on predator-prey interactions between *Amblyseius hibisci* and *Oligonychus punicae* (Acarina: Phytoseiidae, Tetranychidae) under greenhouse conditions. Ann Entomol Soc Am 59:793-800.

Meagher, R.L. and French, J.V. (2004) Augmentation of Parasitoids for Biological Control of Citrus Blackfly in Southern Texas. Fla Entomol 87:186-193.

Meyer, M.K.P. (1987) African Tetranychidae (Acari: Prostigmata) - with reference to the world genera. Entomology Memoir, Department of Agriculture and Water Supply, Republic of South Africa 69:1-175.

Mizell, R.F. and Sconyers, M.C. (1992) Toxicity of imidacloprid to selected arthropod predators in the laboratory. Fla Entomol 75:277-280.

Moraes, G.J. and Flechtmann, C.H.W. (2008) Manual de Acarologia: Acarologia básica e ácaros de plantas cultivadas no Brasil. Holos Editora, Ribeirão Preto.

Nadimi, A., Kamali, K., Arbabi, M. and Abdoli, F. (2008) Side-effects of three acaricides on the predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) under laboratory conditions. Munis Entomol Zool 3:556-567.

Natwick, E.T., Palumbo, J.C. and Engle, C.E. (1996) Effects of imidacloprid on colonization of aphids and silverleaf whitefly and growth, yield and phytotoxicity in cauliflower. Southwestern Entomol 21:283-292.

Nauen, R., Tietjen, K., Wagner, K. and Elbert, A. (1998) Efficacy of plant metabolites of imidacloprid against *Myzus persicae* and *Aphis gossypii* (Homoptera : Aphididae). Pestic Sci 52:53-57.

Nomikou, M., Janssen, A., Schraag, R. and Sabelis, M. (2002) Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. Exp Appl Acarol 27:57-68.

Nyrop, J., English-Loeb, G. and Roda, A. (1998) Conservation biological control of spider mites in perennial cropping systems. *In* Barbosa, P. (ed.) Conservation biological control. San Diego, California (USA).

Ochiai, N., Mizuno, M., Mimori, N., Miyake, T., Dekeyser, M., Canlas, L. and Takeda, M. (2007) Toxicity of bifenazate and its principal active metabolite, diazene, to *Tetranychus urticae, Panonychus citri* and their relative toxicity to the predaceous mites, *Phytoseiulus persimilis* and *Neoseiulus californicus*. Exp Appl Acarol 43:181-197.

Olivero, J., García, E., Wong, E., Márquez, A.L. and García, S. (2003) Defining a method to determine the release dose of *Cryptolaemus montrouzieri* Muls based on the incidence of *Planococcus citri* Risso in Citrus orchards. IOBC/wprs Bull 26:155-162.

Onzo, A., Hanna, R., Zannou, I., Sabelis, M.W. and Yaninek, J.S. (2003) Dynamics of refuge use: diurnal, vertical migration by predatory and herbivorous mites within cassava plants. Oikos 101:59-69.

Ouyang, Y., Grafton-Cardwell, E. and Bugg, R.L. (1992) Effects of various pollens on development, survivorship, and reproduction of *Euseius tularensis* (Acari: Phytoseiidae). Environ Entomol 21:1371-1376.

Overmeer, W.P.J. (1985) Rearing and Handling. *In* Helle, W., and Sabelis, M.W. (ed.) World crop pests, vol. 1B. Spider mites: their biology, natural enemies and control. Elsevier, Amsterdam, The Netherlands. p. 161-169.

Palevsky, E., Gal, S., Maoz, Y., Abrahams, Y., Melamed, E., Domeratzky, S., Gross, S., Shmueli, S., Gan-Mor, S., Ronen, B. and Argov, Y. (2010) Windborne pollen provisioning cover crops (WPPCC) for enhancing the population levels of *Euseius scutalis* and improving citrus rust mite control. IOBC/wprs Bull 62:93-97.

Palevsky, E., Walzer, A., Gal, S. and Schausberger, P. (2008) Evaluation of dry-adapted strains of the predatory mite Neoseiulus californicus for spider mite control on cucumber, strawberry and pepper. Exp Appl Acarol 45:15-27.

Park, Y.L. and Lee, J.H. (2002) Leaf cell and tissue damage of cucumber caused by twospotted spider mite (Acari: Tetranychidae). J Econ Entomol 95:952-957.

Pascual-Ruiz, S. (2005) Efectos secundarios de cinco acaricidas sobre el coccinélido depredador *Cryptolaemus montrouzieri* . Trabajo final de carrera, Universidad Miguel Hernández.

Pérez-Sayas, C., Pina, T., Gómez-Martínez, M., Jacas, J.A., and Hurtado, M.A. (2012) Mite predator-prey relationships in citrus could be disentangled by use of Multiplex PCR. XXIV Congress of Entomomology, Daegu, Korea.

Pina, T. , Argolo, P.S. , Urbaneja, A. and Jacas, J.A. (2012) Effect of pollen quality on the efficacy of two different life-style predatory mites against *Tetranychus urticae* in citrus. Biol Control 61:176-183.

Pina, T., Pérez-Sayas, C., Jacas, J.A, and Hurtado, M.A. (2011) Optimization of the PCR-multiplex technique for prey detection in citrus mites IOBC/wprs Bull 62: 99

Poletti, M., Maia, A.H.N. and Omoto, C. (2007) Toxicity of neonicotinoid insecticides to *Neoseiulus californicus* and *Phytoseiulus macropilis* (Acari: Phytoseiidae) and their impact on functional response to *Tetranychus urticae* (Acari: Tetranychidae). Biol Control 40:30-36.

Popov, N.A. and Khudyakova, O.A. (2008) Development of *Phytoseiulus persimilis* (Acarina: Phytoseiidae) fed on *Tetranychus urticae* (Acarina: Tetranychidae) on various food plants. Acta Entomol Fenica 53:43-46.

Powell, C.A., Burton, M.S., Pelosi, R.R., Rundell, P.A., Ritenour, M.A. and Bullock, R.C. (2006) Six-year evaluation of brown citrus and Spirea aphid populations in a citrus grove and the effects of insecticides on these populations. Hortsci 41:688-690.

Pozzebon, A., Duso, C., Tirello, P. and Ortiz, P.B. (2011) Toxicity of thiamethoxam to *Tetranychus urticae* Koch and *Phytoseiulus persimilis* Athias-Henriot (Acari Tetranychidae, Phytoseiidae) through different routes of exposure. Pest Manag Sci 67:352-359.

Pratt, P.D. (1998) Within- and between-plant dispersal and distributions of *Neoseiulus californicus* and *N. fallacis* (Acari: Phytoseiidae) in simulated bean and Apple plant systems. Environ Entomol 27:148-153.

Pratt, P.D. and Croft, B.A. (2000) Toxicity of pesticides registered for use in landscape nurseries to Acarine biological control agent, *N. fallacis*. J Environ Hort 18:197-201.

Ragusa, E., Tsolakis, H. and Jordà Palomero, R. (2009) Effect of pollens and preys on various biological parameters of the generalist mite *Cydnodromus californicus*. Bull Insectol 62:153-158.

Ramzi, M., Fatma, E.Y., Kaouthar, G.L. and Salah, R. (2010) Imidacloprid applied through drip irrigation as a new promising alternative to control mealybugs in Tunisian vineyards. J Plant Prot Res 50:314-319.

Raworth, D.A. (1986) Sampling statistics and a sampling scheme for the twospotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on strawberries. Can Entomol 118:807-814.

Ripollés, J.L., Caballero, A., Martínez-Ferrer, M.T., Fibra, J.M. and Carulla, R. (1996) Métodos de muestreo para *Phyllocnistis citrella*, el minador de los cítricos. Determinación de la densidad de brotación. Levante Agrícola 337:320-327.

Ripollés, J.L. and Melia, A. (1980) Primeras observaciones sobre la proliferación de *Conwentzia psociformis* (Curt.) (Neuroptera, Coniopterygidae), en los cítricos de Castellón de la Plana. Bol Serv Plagas 6:61-66.

Rodriguez, J.M., García-Marí, F. and Ferragut, F. (1992) Actividad depredadora de varios ácaros fitoseidos sobre distintos estados de desarrollo del trips de las flores *Frankliniella occidentalis*. Bol San Veg Plagas 18:253-263.

Sabelis, M.W. (1981) Biological control of two-spotted spider mites using phytoseiid predators. Part I: modelling the predator-prey interaction at the individual level. Agri Res Reports -242.

San Andrés, V., Abad, R., Ansaloni, T., Aucejo, S., Belliure, B., Dembilio, O., Jacas, J.A., Mora, J., Pascual-Ruiz, S., Pina, T., Ripollés, J.L., Vanaclocha, P. and Urbaneja, A. (2006) Efectos secundarios sobre *Euseius stipulatus* de tratamientos cebo dirigidos al control de *Ceratitis capitata*. Phytoma España 180:38-45.

Sazo, L., Araya, J.E. and Iturriaga, P. (2006) Efecto del tipo de polen sobre la supervivencia, fertilidad y viabilidad de los huevos de *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) en laboratorio. Bol San Veg Plagas 32:619-623.

Schausberger, P. (2003) Cannibalism among phytoseiid mites: a review. Exp Appl Acarol 29:173-191.

Sétamou, M., Rodriguez, D., Saldana, R., Schwarzlose, G., Palrang, D. and Nelson, S.D. (2010) Efficacy and uptake of soil-applied imidacloprid in the control of Asian citrus psyllid and a citrus leafminer, two foliar-feeding citrus pests. J Econ Entomol 103:1711-1719.

Shimoda, T. and Takabayashi, J. (2001) Migration of specialist insect predators to exploit patchily distributed spider mites. Popul Ecol 43:15-21.

Skirvin, D.J. and Fenlon, J.S. (2003) The effect of temperature on the functional response of *Phytoseiulus persimilis* (Acari : Phytoseiidae). Exp Appl Acarol 31:37-49.

Smith, D., Beattie, G.A.C. and Broadley, R. (1997) Citrus pests and their natural enemies. Integrated Pest Management in Australia. Brisbane, Australia. p. 282.

Smith, D. and Papacek, D.F. (1991) Studies of the predatory mite *Amblyseius victoriensis* (Acarina: Phytoseiidae) in citrus orchards in south-east Queensland: control of *Tegolophus australis* and *Phyllocoptruta oleivora* (Acarina: Eriophyidae), effect of pesticides, alternative host plants and augmentative release. Exp Appl Acarol 12:195-217.

So, P.M. (1991) Distribution patterns of and sampling plans for *Tetranychus urticae* Koch (Acarina: Tetranychidae) on roses. Res Popul Ecol 33:229-243.

SPSS Inc, I. C. (2010) IBM® SPSS® Statistic for Windows®, version 19.0.0. New York, USA.

Sterk, G., Hassan, S.A., Baillod, M., Bakker, F., Bigler, F., Blümel, S., Bogenschütz, H., Boller, E., Bromand, B., Brun, J., Calis, J.N.M., Coremans-Pelseneer, J., Duso, C., Garrido, A., Grove, A., Heimbach, U., Hokkanen, H., Jacas, J., Lewis, G., Moreth, L., Polgar, L., Rovesti, L., Samsöe-Petersen, L., Sauphanor, B., Schaub, L., Stäubli, A., Tuset, J. J., Vainio, A., van DeVeire, M., Viggiani, G., Vinuela, E. and Vogt, H. (1999) Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". BioControl 44:99-117.

Straub, C.S. and Snyder, W.E. Species identity dominates the relationship between predator biodiversity and herbivore suppression. Ecology . 2006

Sunnucks, P. and Hales, D.F. (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Mol Biol Evol 13:510-524.

UE (2009) Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. OJEU .

Urbaneja, A., Catalán, J., Tena, A., and Jacas, J.A. (2012) Gestión Integrada de Plagas de Cítricos http://gipcitricos.ivia.es/ Accessed: 1-4-2011

Urbaneja, A., Llácer, E., Tomás, O., Garrido, A. and Jacas, J.A. (2000) Indigenous natural enemies associated with *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Eastern Spain. Biol Control 18:199-207.

Urbaneja, A., Pascual-Ruiz, S., Pina, T., Abad-Moyano, R., Vanaclocha, P., Montón, H., Castañera, P. and Jacas, J.A. (2008) Efficacy of some acaricides against *Tetranychus urticae* (Acari: Tetranychidae) and their side-effects on selected natural enemies occurring in citrus orchards. Pest Manag Sci 64:834-842.

Vacas, S., Vanaclocha, P., Alfaro, C., Primo, J., Verdú, M.J., Urbaneja, A. and Navarro-Llopis, V. (2012) Mating disruption for the control of *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) may contribute to increased effectiveness of natural enemies. Pest Manag Sci 68:142-148.

van de Viere, M. and Tirry, L. (2003) Side effects of pesticides on four species of beneficials used in IPM in glasshouse vegetable crops: "worst case" laboratory tests. IOBC/wprs Bull 26:41-50.

van Driesche, R., Hoddle, M. and Center, T. (2009) Augmentative release of natural enemies in outdoor crops. *In* van Driesche, R., Hoddle, M., and Center, T. (ed.) Control pf Pests and Weeds by Natural Enemies: an Introduction to Biological Control. p. 324-338.

van Lenteren, J.C. and Woets, J. (1988) Biological and Integrated Pest control in Greenhouses. Annu Rev Entomol 33:239-269.

van Lenteren, J.C. (2000) A greenhouse without pesticides: fact or fantasy? Crop Prot 19:375-384.

van Lenteren, J.C. and Woets, J. (1988) Biological and Integrated Pest control in Greenhouses. Annu Rev Entomol 33:239-269.

van Rijn, P.C.J. and Tanigoshi, L.K. (1999) Pollen as food for the predatory mites *Iphiseius degenerans* and *Neoseiulus cucumeris* (Acari: Phytoseiidae): dietary range and life history. Exp Appl Acarol 23:785-802.

van Rijn, P.C.J., van Houten, Y.M. and Sabelis, M.W. (2002) How plants benefit from providing food to predators even when it is also edible to herbivores. Ecology 83:2664-2679.

Vanaclocha, P., Vidal-Quist, C., Oheix, S., Montón, H., Planes, L., Catalán, J., Tena, A., Verdú, M.J. and Urbaneja, A. (2012) Acute toxicity in laboratory tests of fresh and aged residues of pesticides used in citrus on the parasitoid *Aphytis melinus*. J Pest Sci 1-8.

Villanueva, R.T. and Walgenbach, J.F. (2005) Development, oviposition, and mortality of *Neoseiulus fallacis* (Acari: Phytoseiidae) in response to reduced-risk insecticides. J Econ Entomol 98:2114-2120.

Walzer, A., Castagnoli, M., Simoni, S., Liguori, M., Palevsky, E. and Schausberger, P. (2007) Intraspecific variation in humidity susceptibility of the predatory mite *Neoseiulus californicus*: Survival, development and reproduction. Biol Control 41:42-52.

Walzer, A., Moder, K. and Schausberger, P. (2009) Spatiotemporal within-plant distribution of the spider mite *Tetranychus urticae* and associated specialist and generalist predators. Bull Entomol Res 99:457-466.

Wilson, F. (1966) The conservation and augmentation of natural enemies. Int J Pest Manag: Part A 12:40-46.

Yasuyuki, N., Tomoyuki, J. and Hiroshi, N. (2004) Seasonal prevalence of occurrence and the spatial distribution of spider mites and their natural enemies at the apple orchard of AFC Campus Station. Bull Shin Univ Alp Field Center 2:23-30.

Zhang, Z.Q. and Sanderson, J.P. (1990) Relative Toxicity of Abamectin to the Predatory Mite *Phytoseiulus persimilis* (Acari: Phytoseiidae) and Two spotted Spider Mite (Acari: Tetranychidae). J Econ Entomol 83:1783-1790.

Zhang, Z.Q. (2003) Mites of Greenhouses: Identification, Biology and Control. CABI Publishing (ed.) 244 pp Wallingford, UK.

Zhang, Z.Q. and Jacobson, R.J. (2000) Using adult female morphological characters for differentiating *Tetranychus urticae* complex (Acari: Tetranychidae) from greenhouse tomato crops in UK. Syst Appl Acarol 5:69-76.

Zhang, Z.Q., Sanderson, J.P. and Nyrop, J.P. (1992) Foraging time and spatial patterns of predation in experimental populations. Oecologia 90:185-196.

Zhimo, Z. and McMurtry, J.A. (1990) Development and reproduction of three *Euseius* (Acari: Phytoseiidae) species in the presence and absence of supplementary foods. Exp Appl Acarol 8:233-242.