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

Laura Planes[1], *Jose Catalán*[1], *Josep A. Jaques*[2b], *Alberto Urbaneja*[1], *and Alejandro Tena*[1*]

Abstract

Kelly's citrus thrips *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) is a new pest of oranges in New Zealand, southern Australia, and the Mediterranean Basin. The nymphs of this thrips can damage the fruit from petal fall up to 6 wk later. Because there is a lack of information on its management, the aims of this study were to determine the number of generations occurring on the fruit and the efficacy of 3 insecticides (chlorpyrifos, spinosad, and spirotetramat) to control this pest. Chlorpyrifos and spinosad displayed a high efficacy against nymphs and reduced significantly the percentage of damaged fruit when a single generation of *P. kellyanus* attacked the fruit. However, these insecticides did not prevent development of a subsequent generation of *P. kellyanus*. The percentage of damaged fruit was higher when the 2nd generation was present. Spirotetramat did not display a knockdown effect, and its efficacy was less than that of chlorpyrifos and spinosad. Similar to these insecticides, spirotetramat did not prevent the attack of a 2nd generation when it occurred. Additionally, we analyzed the side effects of these treatments on predatory mites. Spinosad and spirotetramat negatively affected these beneficial species.

Key Words: citrus; IPM; chlorpyriphos; spinosad; spirotetramat; side effect; predatory mite

Resumen

Pezothrips kellyanus (Bagnall) (Thysanoptera: Thripidae) es una nueva plaga de cítricos en Nueva Zelanda, el sur de Australia y la Cuenca del Mediterráneo. Las ninfas de estos trips pueden dañar los frutos desde la caída de pétalos hasta sesi semanas después. A pesar de los estudios realizados sobre esta plaga y que hasta ahora sólo es posible su control mediante insecticidas, la información para realizar un adecuado manejo integrado es insuficiente. Por ello, el objetivo de este estudio fue determinar el número de generaciones que pueden encontrarse sobre el fruto y la eficacia de tres insecticidas (clorpirifos, spinosad y spirotetramat) con diferente modo de acción en el control de esta plaga y los efectos secundarios sobre fitoseidos en cítricos. Clorpirifos y spionsad tuvieron una elevada eficacia contra ninfas y redujeron significativamente el porcentaje de frutos dañados cuando fueron atacados por una sola generación. Sin embargo, su actividad no pudo prevenir el ataque de una siguiente generación de *P. kellyanus*. Además, el pordentaje de frutos dañados aumentó cuando se detectó esta segunda generación. Spirotetramat no tuvo un efecto de choque y su eficacia fue menor que la de clorpirifos y spinosad. Al igual que estos insecticidas, spirotetramat no pudo evitar un segundo ataque cuando se produjo. Los efectos secundarios evaluados de estos tratamientos sobre fitoseidos mostraron que spinosad y spirotetramat les afectaron negativamente.

Palabras Clave: cítricos; MIP; clorpirifos; spinosad; spirotetramat; efectos secundarios; fitoseidos

Kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae), is a new pest of citrus (Stevens et al. 1998; Webster et al. 2006; Vassiliou 2007; Navarro et al. 2008). It became a pest in New Zealand (Blank & Gill 1997) and southern Australia (Mound & Jackman 1998) during the 1990s. In the Mediterranean Region, the first damage caused by *P. kellyanus* was recorded a few years later, and now this thrips is considered a pest in Greece (Varikou et al. 2010), Cyprus (Vassiliou 2007), Sicily (Italy) (Marullo 1998; Conti et al. 2003), and Spain (Navarro-Campos et al. 2012a). *Pezothrips kellyanus* nymphs feed on the surface of young citrus fruits for 5 to 6 wk starting at petal fall (Navarro-Campos et al. 2013). This feeding habit causes patches or rings of scarred tissue around the fruit apex that enlarge as the fruit grows. This damage is particularly severe on navel orange, lemon (Conti et al. 2003), and grapefruit (Mound & Jackman 1998; Baker et al. 2004; Vassiliou 2007, 2010). Although feeding damage does not affect the internal quality of the fruit, this damage leads to economic losses due to reduced market value of the affected fruits. The percentage of citrus fruits with a complete ring scar may reach 70% per orchard (Varikou 2002; Vassiliou 2010).

Despite the worldwide distribution and economic importance of *P. kellyanus*, its biological control is still under development (Baker et al. 2011; Navarro-Campos et al. 2012a). Therefore, chemical control is currently the only practical alternative for growers. However, its implementation, results, and side effects are poorly known. First of

---

[1] Instituto Valenciano de Investigaciones Agrarias, IVIA; Unidad Asociada de Entomología UJI-IVIA. Centro de Protección Vegetal y Biotecnología; Ctra. de Moncada a Náquera km 4.5; E-46113, Moncada, Spain

[2] Universitat Jaume I, UJI; Unitat Associada d'Entomologia UJI-IVIA; Departament de Ciències Agràries i del Medi Natural; Campus del Riu Sec; E-12071, Castelló de la Plana, Spain

*Corresponding author; E-mail: atena@ivia.es

[b] Formerly Josep A. Jacas

all, the number of treatments necessary to reduce thrips populations is unclear. From 1 to 3 insecticide applications are directed to these pests (Conti et al. 2004; Vassiliou 2007). Second, the efficacy of insecticides on *P. kellyanus* nymphs, the stage that produces the damage, has never been determined. The efficacy of the treatments has been determined based on fruit damage, but whether these applications reduce either the 1st or the 2nd generations of thrips, or both, remains unknown. Third, the side effects of these treatments on the natural enemies of other important citrus pests have not been studied. Importantly, insecticides are sprayed in spring, when the populations of key natural enemies are increasing after winter (Martínez-Ferrer 2007; Tena et al. 2008; Urbaneja et al. 2008, 2009; Sorribas & Garcia-Marí 2010). These natural enemies are responsible for the excellent biological control of many occasional and secondary citrus pests on orange cultivars in Spain (Jacas & Urbaneja 2009). Finally, *P. kellyanus* may develop resistance to insecticides if its chemical control relies on only a single class of insecticides (Baker et al. 2004).

Therefore, it is important to determine the efficacy against *P. kellyanus* of insecticides with different modes of action. Chlorpyrifos, an organophosphate insecticide, is one of the most-widely used insecticides for pest control in citrus against hemipterans (scales and aphids) and thrips (Morse & Grafton-Cardwell 2012a; Navarro-Campos et al. 2012b; Planes et al. 2013). It is used against the latter because of its fast-acting effect. However, its persistence against more than 1 generation of *P. kellyanus* is unknown. Spinosad, a mixture of tetracyclic-macrolide compounds, has been identified as potential candidate for integrated pest management (IPM) programs in citrus because of its fast action (insects dying of exhaustion within 1–2 d) and its low persistence (Thompson et al. 2000; Cisneros et al. 2002). Its residues on the leaf surface are degraded by sunlight within a few days (Salgado 1998). Because of these characteristics, spinosad is recommended in citrus against *Scirtothrips citri* (Moulton) (Thysanoptera: Thripidae) in California (Immaraju et al. 1989; Khan & Morse 2006; Morse & Grafton-Cardwell 2012b). Spirotetramat is a new systemic and persistent foliar insecticide. It is a tetramic acid derivative with a novel mode of action that interferes with lipid biosynthesis, leading to the death of immature stages of the target insect 2 to 10 d after application (IRAC 2014). Spirotetramat is active against a wide spectrum of sucking insects, including aphids, scales (soft and armored), mealybugs, whiteflies, psyllids, and selected thrips species (Grafton-Cardwell et al. 2007). Therefore, it could be used against *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) and *P. kellyanus* with a single application at the end of spring. Moreover, its long persistence could make it active against a possible 2nd generation of *P. kellyanus*.

In this study, we determined: i) the efficacy of 3 insecticides with different modes of action (chlorpyrifos, spinosad, and spirotetramat) against nymphs and adults of *P. kellyanus* in the field; ii) their persistence against subsequent generations of this thrips; and iii) their effectiveness in decreasing the percentage of damaged fruit. These results would allow us to make an educated recommendation about the number of treatments necessary when these insecticides are used. Finally, iv) we also determined the side effects of these treatments on phytoseiid mite predators, one of the key group of natural enemies in citrus.

Materials and Methods

INSECTICIDES

The insecticides evaluated were chlorpyrifos, , and spirotetramat (Table 1). Following the recommendations of IPM for citrus (Urbaneja et al. 2013), insecticides were applied in the morning, when conditions were calm. The concentrations of the commercial products tested in these assays were the maximum authorized in citrus in Spain. For spirotetramat, the concentrations used were recommended by the technical department of Bayer Crop Science (Valencia, Spain). Insecticides were applied when the percentage of occupied fruits was above the economic injury level set at 7% occupied fruits (Navarro-Campos et al. 2012b). For this purpose, orchards were sampled weekly for 5 to 6 wk starting at petal fall.

FIELD ASSAYS

Alzira Orchard

This assay was conducted in a 16-yr-old navel orange 'Lane-late' orchard (*Citrus sinensis* Blanco var. Navel Lane-Late grafted on Citrange 'Carrizo' [*Citrus sinensis* L. Osbeck × *Poncirus trifoliata* Blanco]; Sapindales: Rutaceae) located near the town Alzira (39°08ˈ59"N, 0°25ˈ59"W) (Valencia, Spain) in 2010. The orchard had 1.4 ha and the planting pattern was 6 × 5 m. It was drip irrigated and the naturally occurring cover crop was mowed annually at the beginning of spring. The population density of nymphs was above the economic threshold at petal fall (25 May). On this day, 35 trees were sampled and selected according to their similar infestation level by *P. kellyanus* nymphs (15–25% occupied fruits). On the next day, insecticides were applied with a hand gun, using outside coverage with a volume of about 4.5 L per tree (approx. 1,500 L/ha). Ten, 8, and 8 trees (replicates) were sprayed with chlorpyrifos, spinosad, and spirotetramat, respectively, and the remaining 9 trees were not treated and served as controls. To avoid possible interferences, the 8 trees surrounding every treated tree received the same treatment.

To monitor thrips populations and determine the efficacy of the insecticides, we sampled 32 fruits (8 per orientation) per tree 1 d prior to the spray, 2 d later, and then weekly until the end of the study. On each fruit, we determined the presence of *P. kellyanus* nymphs. Insecticide efficacy was calculated using Abbott's formula (Abbott 1925). The percentage of damaged fruit was determined on 22 Nov in the same trees. We sampled 40 fruits per tree for damage assessment and distinguished between slightly and severely damaged fruits. We considered severely damaged fruits to be those that had complete ring-like scars and slightly damaged ones to be those with incomplete ring-like scars.

Tavernes Orchard

This assay was conducted in a 10-yr-old navel orange 'Lane-late' orchard (*Citrus sinensis* Blanco var. Navel Lane-Late grafted on Cleopatra mandarin [*Citrus reshni* Hort. ex. Tan.]; Sapindales: Rutaceae) located near the town Tavernes de la Valldigna (39°4ˈ20"N, 0°15ˈ57"W) (Valencia, Spain) in 2010. The orchard had 3.5 ha and the planting pattern was

Table 1. Insecticides used in the assays.

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

6 × 4 m. It was drip irrigated and the naturally occurring cover crop was mowed annually at the beginning of spring. The experimental design was a randomized block with 4 replicates of 4 treatments. Each replicate contained 3 rows of 16 to 30 trees. The population density of nymphs exceeded the economic threshold 2 wk after petal fall (31 May). On this day, 10 trees from each central row were labeled and sampled. One day later, 1,500 L/ha were applied with an air blast sprayer at 30 atm of pressure (Fede mod. Select dynamic; Fede S. L.; Cheste, Spain) to achieve outside tree coverage as is normal for citrus aphids–thrips treatments (Chueca et al. 2009). To follow thrips populations and determine the efficacy of the insecticides, we sampled 32 fruits per tree (8 per orientation) the day prior to the spray, 2 d later, and then weekly until the end of the study. We determined the presence of *P. kellyanus* nymphs on each fruit. On 23 Nov, the percentage of damaged fruit was determined as above.

To determine the population trends of *P. kellyanus* adults and natural enemies of citrus pests under the different insecticide treatments, a portable, engine-powered suction device was used to collect all arthropods (Tena et al. 2008). The device was constructed by modifying a commercial vacuum-blower (Husqvarna Zenoah Co., model HBZ2601, Japan) adapted to collect insects from the foliage. We modified it by adding a cylindrical plastic pipe 50 cm long with a 30 cm diameter opening. The sampling was standardized by placing the opening of the cylindrical pipe 4 times, for 5 to 8 s each time, on the foliage of 10 citrus trees per date and tree (40 times in total). We sampled 10 trees from the central row in each replicate (4 replicates per treatment). The material collected was bagged and transported to the laboratory, where it was held at −20 °C to kill all insects. Adult thrips and natural enemies were counted and identified up to genus or species level under a binocular microscope. Insecticide efficacy on *P. kellyanus* adults was calculated using Abbott's formula (Abbott 1925).

We also determined the side effects of the selected insecticides (Table 1) on phytoseiid abundance. We counted the number of live phytoseiids on the underside of 5 interior and mature leaves per tree. Leaves were randomly selected in the canopies of the same trees sampled for *P. kellyanus*. The mean number of phytoseiids per leaf was determined for each block and treatment on each date sampled. Cumulative phytoseiid-days per leaf were calculated as an index of phytoseiid population for each replicate as:

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

Where ∑ is summation over all sampling dates from the 1st evaluated day, on 31 May, to the last one, on 13 Jul; I_t is the interval between two successive sampling dates; and x_i and x_j are phytoseiid densities on those dates (Hardman et al. 2006; Kahn & Morse 2006).

STATISTICAL ANALYSES

Datasets were first tested for normality and homogeneity of variance using Kolmogorov–Smirnov and Cochran's tests, respectively, and transformed (angular transformation for percentage data) if needed. Subsequently, 1-way ANOVA followed by Tukey post hoc tests for multiple comparisons inside the different application time sub-datasets were carried out for dates and locations with statistically significant differences, or nearly significant differences.

Results

EFFICACY AGAINST *P. KELLYANUS* NYMPHS

The percentage of fruits occupied by *P. kellyanus* nymphs exceeded the economic thresholds (7%) at petal fall at the orchard in Alzira, and 1 wk after the petal fall at Tavernes. This percentage was similar in all the treatments in both orchards (Table 2).

Table 2. Occupancy and insecticide efficacy (when occupancy was significantly different from the control) of fruits by *Pezothrips kellyanus* in 2 orchards located in Alzira and Tavernes.

		Day −1	Day 2		Day 7		Day 14	Day 21	Day 28
Orchard	Treatments	% occupancy	% occupancy	% efficacy	% occupancy	% efficacy	% occupancy	% occupancy	% occupancy
Alzira	Control	20.00 ± 1.50	19.72 ± 2.45 a		1.61 ± 0.56 a		2.22 ± 0.90	2.40 ± 0.33	1.61 ± 0.56
	Chlorpyrifos	22.25 ± 1.77	0.75 ± 0.38 c	96.20 ± 1.90 a	1.70 ± 0.39 a		2.75 ± 0.95	2.26 ± 0.36	1.70 ± 0.39
	Spinosad	18.13 ± 2.25	0.0 ± 0.0 c	98.42 ± 1.58 a	1.62 ± 0.34 a		0.31 ± 0.31	1.54 ± 0.26	1.62 ± 0.34
	Spirotetramat	20.63 ± 1.75	9.06 ± 1.70 b	54.05 ± 8.60 b	1.41 ± 0.31 a		1.56 ± 0.66	1.67 ± 0.32	1.41 ± 0.31
		$F_{3,34} = 0.88$ $P = 3.34$	$F_{3,34} = 38.12$ $P < 0.001$	$F_{2,25} = 27.39$ $P < 0.001$	$F_{3,34} = 0.09$ $P = 0.96$		$F_{3,34} = 1.79$ $P = 0.17$	$F_{3,34} = 1.76$ $P = 0.21$	$F_{3,34} = 0.09$ $P = 0.96$
Tavernes	Control	15.00 ± 1.15	10.16 ± 0.53 a		2.44 ± 0.67 a		2.03 ± 0.70	5.46 ± 1.47	6.09 ± 0.82
	Chlorpyrifos	13.44 ± 1.81	0.55 ± 0.15 b	94.62 ± 1.47	0.63 ± 0.13 b	74.19 ± 5.17	1.09 ± 0.53	5.46 ± 1.13	8.59 ± 0.86
	Spinosad	14.53 ± 3.04	1.02 ± 0.45 b	90.00 ± 4.42	0.40 ± 0.24 b	83.26 ± 9.88	0.96 ± 0.45	5.49 ± 1.48	7.69 ± 1.42
	Spirotetramat	14.90 ± 0.77	8.38 ± 3.00 a		1.64 ± 0.34 ab		1.44 ± 1.03	7.53 ± 3.02	5.61 ± 1.65
		$F_{3,15} = 0.14$ $P = 0.94$	$F_{3,15} = 10.15$ $P = 0.001$	$F_{1,7} = 0.18$ $P = 0.68$	$F_{3,15} = 5.51$ $P = 0.01$	$F_{1,7} = 1.40$ $P = 0.28$	$F_{3,15} = 0.43$ $P = 0.73$	$F_{3,15} = 0.25$ $P = 0.86$	$F_{3,15} = 1.11$ $P = 0.38$

Data presented are mean % ± SE. Means followed by different lowercase letters within a column and location are significantly different (1-way ANOVA followed by Tukey post hoc tests, $P < 0.05$).

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

In Tavernes, the percentage of occupied fruits was significantly higher in control and spirotetramat plots than in spinosad and chlorpyrifos plots 2 and 7 d after the treatment (Table 2). The efficacy of spinosad and chlorpyrifos was high, and there were no significant differences between them on both sampling days. Fourteen days after the treatment, the percentage of occupied fruits decreased, and there were no significant differences among treatments. Twenty-one days after the treatments, the percentage of occupied fruits increased again and remained close to the economic thresholds for the following weeks in all the treatments. On 8 Jul, the orchard was treated with chlorpyrifos, and *P. kellyanus* populations decreased.

EFFICACY AGAINST *P. KELLYANUS* ADULTS

Of the 2,275 adult thrips collected with the vacuum device in Tavernes, 1,951 (85.8%) were *P. kellyanus.* The number of *P. kellyanus* adults captured 1 d before the treatments was similar among treatments ($F_{3, 15} = 1.87$; $P = 0.19$) (Fig. 1). However, 2 d after the treatments, the number of adults increased and became significantly greater in control plots and in plots treated with spirotetramat ($F_{3, 15} = 8.59$; $P = 0.002$) than in the other treatments. The efficacy of spinosad (89.4 ± 4.1 %, mean ± SE) and chlorpyrifos (86.5 ± 4.4 %) was high, and there were no significant differences between them ($F_{1, 7} = 0.22$; $P = 0.65$). Seven days after the treatments, the numbers of captured adults remained significantly smaller than in the control only in the plots treated with spinosad ($F_{3, 15} = 5.33$; $P = 0.015$). Fourteen days after the treatment, the numbers of captured adults decreased in the control plots and were the same in all treated plots ($F_{3, 15} = 0.68$; $P = 0.58$).

DAMAGE

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

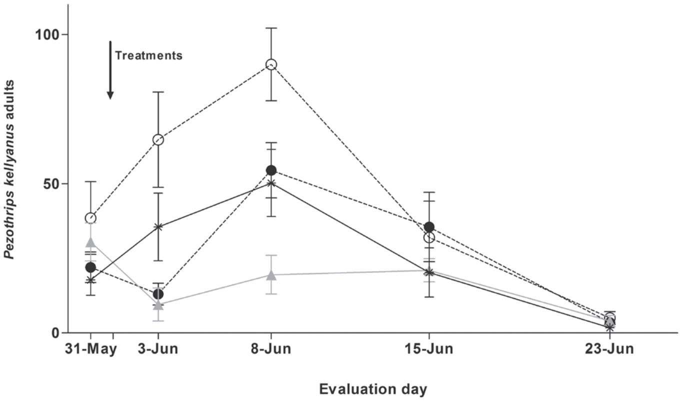

Fig. 1. Number of *Pezothrips kellyanus* adults collected with a vacuum device in a navel orchard located in Tavernes (mean ± SE). Trees were treated with chlorpyrifos, spinosad, or spirotetramat.

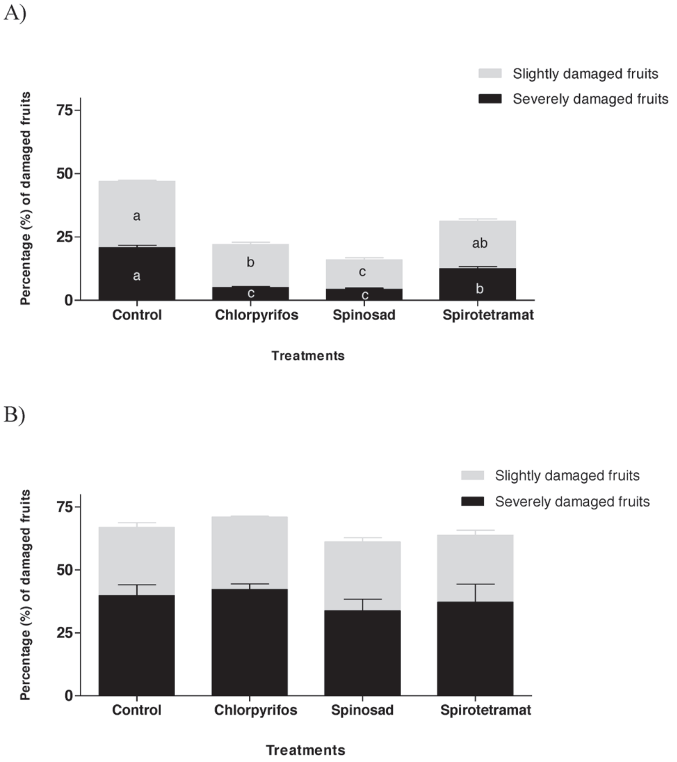

Fig. 2. Percentage (mean ± SE) of fruits slightly and severely damaged by *Pezothrips kellyanus* nymphs in every insecticide plot in orchards of Alzira (A) and Tavernes (B). Trees were treated with chlorpyrifos, spinosad, or spirotetramat. Different letters indicate significant differences ($P < 0.05$) between treatments (1-way ANOVA followed by Tukey post hoc tests).

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

SIDE EFFECTS

The numbers of phytoseiids per leaf were similar in all plots the day before treatments in Tavernes (Table 3). Their densities did not differ significantly the following days. However, the accumulated phytoseiid-day values, used as an overall summary statistic, were significantly lower in the plots treated with spinosad and spirotetramat than in those untreated or treated with chlorpyrifos.

We captured and identified 1,740 natural enemies with the vacuum device (Table 4). Hymenopteran parasitoids were the most abundant, in total 927 were collected, followed by neuropteran predators (286) and arachnid predators (241). In general, the total number of natural enemies captured was higher in untreated plots (control) than in the treated plots in the following days. There were no significant differences among treatments on day −1 ($F_{3, 15} = 0.85$; $P = 0.49$), day 14 ($F_{3, 15} = 0.50$; $P = 0.69$), and day 21 ($F_{3, 15} = 0.85$; $P = 0.49$) in the total number of natural enemies captured. However, the total numbers of natural enemies captured in the plots treated with spinosad and chlorpyrifos were significantly smaller than in those untreated on day 2 ($F_{3, 15} = 6.27$; $P = 0.0084$). Similarly, the total numbers of natural enemies captured in the plots treated with the 3 insecticides were significantly smaller

Table 3. Side effects of insecticides used against *Pezothrips kellyanus* on phytoseiid populations.

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

Data presented are numbers of phytoseiids and cumulative phytoseiid-days per leaf for each treatment. Means followed by different lowercase letters within a column are significantly different (1-way ANOVA followed by Tukey post hoc tests, $P < 0.05$).

than in those untreated on day 7 ($F_{3,15} = 10.71$; $P = 0.001$). We could not determine the side effects of the insecticides on the main natural enemies of citrus, namely the hymenopteran parasitoids *Aphytis melinus* DeBach (Aphelinidae), *Cales noacki* Howard (Aphelinidae), *Citrostichus phyllocnistoides* (Narayanan) (Eulophidae), and *Metaphycus* spp. (Encyrtidae) and the predators of the family Coccinellidae, because of the small numbers of specimens of these species collected (Table 4).

Discussion

Our results, based on weekly monitoring of *P. kellyanus* immature populations on fruit, indicated that a single insecticide application of either chlorpyrifos or spinosad can suppress *P. kellyanus* nymphs when only 1 generation of *P. kellyanus* attacks the fruit. This was the case of the assay in Alzira. Both chlorpyrifos and spinosad displayed a knockdown effect against *P. kellyanus* nymphs and, 2 d after the treatment, reduced the percentage of occupied fruits below economic thresholds. Both pesticides reached efficacies higher than 90% mortality of the thrips populations. Afterwards, thrips populations remained low in both treated and untreated trees. Thus, only 1 generation attacked the fruit in this assay. As a consequence of this attack, the percentage of damaged fruit at harvest was less than 25% in the trees treated with chlorpyrifos or spinosad, whereas it reached almost 50% in untreated trees. Therefore, these insecticides were able to reduce the abundance of *P. kellyanus* and its damage with a single application when only 1 generation of this thrips attacked the fruit. Both pesticides had been tested previously against this pest with similar results (Benfatto et al. 2000; Purvis et al. 2002). Baker et al. (2004) considered spinosad to be a potential candidate for IPM of *P. kellyanus* in Australia, and Vassiliou (2007) identified chlorpyrifos as the most effective insecticide among 15 tested in his study. The results of these 2 studies were based on the observation of damaged fruit at harvest, though they did not monitor *P. kellyanus* populations before or after the treatments.

By contrast, our assay in Tavernes showed that a single application of chlorpyrifos or spinosad could not suppress a 2nd generation of *P. kellyanus*. As in Alzira, both insecticides reduced the percentage of occupied fruits to below economic thresholds 2 d after treatment. These percentages remained low for 21 d after the treatments, when a new generation of nymphs attacked the fruit in all blocks. Thus, the persistence of chlorpyrifos and spinosad applied against the 1st generation was not enough to control the 2nd one. Consequently, a 2nd treatment would have been necessary to suppress it. Although the percentage of occupied fruits was only one-third as large as the 1st generation, and fruits were larger, this 2nd application seems necessary because the percentage of damaged fruit was very high (above 50%) in this assay. Importantly, all insecticides applied against the 1st generation were able to reduce the percentage of damaged fruit when compared with the control. Vassiliou (2007) sprayed twice against *P. kellyanus*, but the percentage of damaged fruit was approximately 70%. Therefore, a 2nd application does not guarantee a reduction of damaged fruits. In his assay, Vassiliou (2007) did not monitor *P. kellyanus* populations, and it is not known whether the application timing was correct. Consequently, this 2nd application can be recommended only when thrips populations are monitored. Finally, if a 2nd treatment is necessary, the insecticides used should be different from those used against the 1st generation to help avoid development of resistance and to assure continued effectiveness of the available pesticides.

Some populations of citrus thrips have developed resistance to pesticides (Morse & Brawner 1986; Immaraju et al. 1989). In Californian citrus, *S. citri* developed resistance to a long list of insecticides (Morse & Brawner 1986; Immaraju et al. 1989; Khan & Morse 1998). Baker et al. (2004) found that some *P. kellyanus* populations in south-

Table 4. Side effects of insecticides used against *Pezothrips kellyanus* on natural enemies evaluated by total number of insects captured with a vacuum device per treatment and evaluation day.

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

Cont: control; Chlor: chlorpyrifos; Spin: spinosad; Spiro: spirotetramat.

ern Australia had substantial levels of chlorpyrifos resistance. In Spain, chlorpyrifos has been used widely to control *A. aurantii* and other armored scales during the last 2 decades. The high efficacy obtained with chlorpyrifos in our assays suggests that Spanish populations of *P. kellyanus* have not yet developed resistance to this insecticide. To avoid development of resistance, citrus growers should avoid applying chlorpyrifos against both generations of *P. kellyanus* or against *A. aurantii* and *P. kellyanus* within the same year. The most obvious way of delaying the development of resistance to insecticides is to use them only when required, relying whenever possible on other methods of control that are included in IPM programs (Morse & Brawner 1986). In Spain, growers spray twice during this period to protect the fruit from *P. kellyanus* scarring. The 1st treatment is generally applied at petal fall and the 2nd one is usually applied 15 d later as a routine. However, insecticides should not be applied at petal fall as a habitual practice because the 1st generation of thrips may appear later, as occurred in the Tavernes assay. Furthermore, the existence of a 2nd generation may vary among years and locations. In fact, we did not observe a 2nd generation of nymphs in this orchard in 2012 (pers. observations). Consequently, population monitoring is critical to determine the optimum spray timing, assure the efficacy of treatments, and delay the appearance of resistant populations. In California, timing is considered vital to achieve adequate control of *S. citri* with a single application of a relatively short residual pesticide, so that destruction of beneficial organisms is minimized (Morse & Brawner 1986; Morse et al. 1988).

Spirotetramat has been registered recently against *A. aurantii*, *Panonychus citri* (McGregor) (Prostigmata: Tetranychidae), and thrips (Grafton-Cardwell et al. 2007; Grafton-Cardwell & Scott 2008; Morse & Grafton-Cardwell 2009; MAGRAMA 2014). Spirotetramat might be an especially useful insecticide as it could control *A. aurantii* and *P. kellyanus* with a single application in spring. In our study, spirotetramat showed an efficacy around 60% and reduced both slight and severe scarring around 40% relative to control at Alzira in 2009. However, its efficacy was lower than that of chlorpyrifos and spinosad. Also, it did not display a knockdown effect, likely because its contact efficacy is rather limited (Nauen et al. 2008). Thus, its effect on *P. kellyanus* seems to be limited when compared with spinosad and chlorpyrifos. Moreover, despite its systemic and translaminar activity (Nauen et al. 2008), it did not prevent the attack of the 2nd generation in Tavernes.

To avoid the disruption of the excellent biological control of some important orange pests (Jacas & Urbaneja 2009), the insecticides selected for use against *P. kellyanus* should have relatively short residual effects, so their impact on beneficial organisms will be minimized (Morse & Brawner 1986; Morse et al. 1988). This is especially important because *P. kellyanus* is treated at the end of spring when most natural enemy populations are increasing in Spanish citrus (Martínez-Ferrer 2003; Tena et al. 2008; Urbaneja et al. 2008, 2009; Sorribas & Garcia-Marí 2010).

Spinosad and spirotetramat decreased the number of cumulative phytoseiid-days. Spinosad also reduced the number of cumulative phytoseiids-days in a similar study carried out in California (Kahn & Morse 2006). Although spinosad and spirotetramat showed low toxicity in our study, more detailed study is needed to clarify the side effects of spinosad on phytoseiids because it was highly effective against *P. kellyanus* and is therefore a candidate to be used within IPM programs. This is especially relevant if 2 treatments are necessary to control *P. kellyanus*.

In addition to counting phytoseiids, we also collected beneficial insects with a vacuum device. Our data showed that the 3 insecticides had a negative effect on the total number of beneficial insects, and this detrimental effect lasted 1 wk. However, we could not determine the side effects of the insecticides tested on the other 2 groups of natural enemies that are key to Spanish IPM programs in citrus, namely coccinellid predators and hymenopteran parasitoids (Urbaneja et al. 2008). Therefore, we would recommend determining the side effects on representative parasitoids and coccinellids of citrus IPM programs under laboratory conditions to ascertain the actual impact. Some of these studies have already demonstrated that chlorpyrifos is harmful to the parasitoid *A. melinus* (González-Zamora et al. 2013; Vanaclocha et al. 2013) and the coccinellid *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) (Planes et al. 2013).

In conclusion, our study shows that chlorpyrifos and spinosad display a knockdown effect and can control the 1st generation of *P. kellyanus* in citrus with a single application. However, their persistence is not enough to avoid a 2nd generation when it occurs. Therefore, an additional application might be necessary in those cases where this 2nd generation occurs. However, this 2nd application, as the 1st, is justified only when thrips populations are correctly monitored and exceed the potential damage threshold. Finally, because IPM programs on navel oranges in Spain are based on biological control of most of their pests, the development of alternative control strategies to avoid the disruption of the established biological control is urgently needed.

Acknowledgments

We are grateful to Bautista Domènech and Bernardo Villalba for allowing us to sample their orchards. We also thank P. Bru, H. Montón, C. Monzó, B. Sabater, E. Llácer, Khaled, and F. Gómez-Marco (IVIA) for their technical assistance and J. Izquierdo (Bayer Crop Science, Spain) for his collaboration. L. Planes was a recipient of a fellowship from IVIA. A. Tena was a recipient of a postdoctoral fellowship from the MCINN (Juan de la Cierva program). This research was partly funded by the Spanish Ministry of Science and Innovation (project: AGL2008-05287-C04/AGR) and by the Conselleria d'Agricultura, Pesca i Alimentació from Generalitat Valenciana.

References Cited

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

Baker G, Keller M, Purvis S, Jackman D, Crips P. 2004. Improving the management of Kelly's citrus thrips in citrus: summary, conclusions and recommendations of the years 2000-04. http://www.sardi.sa.gov.au/pestsdiseases/horticulture/horticultural_pests/kelly_citrus_thrips/research_report_2000-2004 (last accessed 15 Jan 2014).

Baker GJ, Keller M, Crisp P, Jackman DJ, Barbour D, Purvis S. 2011.The biological control of Kelly's citrus thrips, in Australian citrus orchards. IOBC-WPRS Bulletin 62: 267-274.

Benfatto D, Conti F, Frittitta C, Perrotta G, Raciti E, Tumminelli R. 2000. Risultati di prove di lotta contro il nuovo tripide degli agrumi *Pezothrips kellyanus* (Bagnall). Atti Giornate Fitopatologiche 1: 381-386.

Blank RH, Gill GSC. 1997. Thrips (Thysanoptera: Terebrantia) on flowers and fruit of citrus in New Zealand. New Zealand Journal of Crop and Horticultural Science 25: 319-332.

Chueca P, Garcerá C, Moltó E. 2010. Optimización de las dosis de aplicación de fitosanitarios en tratamientos mecanizados. Vida Rural 317: 23-28.

Cisneros J, Goulson D, Derwent LC, Penagos DI, Hernández O, Williams T. 2002. Toxic effects of spinosad on predatory insects. Biological Control 123: 156-163.

Conti F, Tumminelli R, Fisicaro R, Perrotta G, Marullo R, Liotta G. 2003. An IPM system for new citrus thrips in Italy. IOBC/WPRS Bulletin 26: 203-208.

Conti F, Perrotta G, Colazza S, Maltese U, Azzaro F. 2004. Efficacy and selectivity of pesticides on citrus thrips *Pezothrips kellyanus* Bagnall Thysanoptera: Thripidae. 2004. Oral presentation. Montesilvano Pescara, 4-6 maggio. Atti Giornate Fitopatologiche 1: 59-64.

González-Zamora JE, Castillo ML, Avilla C. 2013. Side effects of different pesticides used in citrus on the adult stage of the parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) and its progeny. Spanish Journal of Agricultural Research 11: 494-504.

Grafton-Cardwell EE, Scott SJ. 2008. Efficacy of acaricides for control of citrus red mite. Arthropod Management Tests 33 (D5).

Grafton-Cardwell EE, Reagan CA, Haviland DR. 2007. Efficacy of Movento to control California red scale. Arthropod Management Tests 32 (D6).

Hardman JM, Franklin JL, Jensen KIN, Moreau DL. 2006. Effects of pesticides on mite predators (Acari: Phytoseiidae) and colonization of apple trees by *Tetranychus urticae*. Phytoparasitica 34: 449-462.

Immaraju J, Morse J, Kersten D. 1989. Citrus thrips (Thysanoptera: Thripidae) pesticide resistance in the Coachella and San Joaquin Valleys of California. Journal of Economic Entomology 82: 374-380.

IRAC. 2014. Resistance management for sustainable agriculture and improved public health. http://www.irac-online.org (last accessed 20 Jan 2014).

Jacas JA, Urbaneja A. 2009. Control Biológico de Plagas Agrícolas. Phytoma-España S.L., Valencia, Spain. 496 pp.

Khan I, Morse JG. 1998. Citrus thrips (Thysanoptera: Thripidae) resistance monitoring in California. Journal of Economic Entomology 91: 361-366.

Khan I, Morse JG. 2006. Impact of citrus thrips treatments on the predatory mite *Euseis tularensis*. Journal of Applied Entomology 130: 386-392.

MAGRAMA. 2014. Ministerio de Agricultura, Alimentación y Medio Ambiente. Registro de productos fitosanitarios. http://www.magrama.gob.es/es/agricultura/temas/sanidad-vegetal/productos-fitosanitarios/registro/productos/consusact.asp (last accessed 15 Jan 2014).

Martínez-Ferrer MT. 2003. Biología y control del cotonet *Planococcus citri* (Homoptera: Pseudococcidae) en huertos de cítricos. Ph.D. thesis. Universidad Politécnica de Valencia, Spain.

Marullo R. 1998. *Pezothrips kellyanus*, un nuovo tripide parassita delle colture meridionali. Informatore Fitopatologico 10: 72-74.

Morse J, Brawner OL. 1986. Toxicity of pesticides to *Scirtothrips citri* (Thysanoptera: Thripidae) and implications to resistance management. Journal of Economic Entomology 79: 565-570.

Morse JG, Grafton-Cardwell EE. 2009. Managing insecticide resistance will be key to the future of effective citrus pest management. Topics in Subtropics 7: 6-8.

Morse JG, Grafton-Cardwell. 2012a. The evolution of biologically-based integrated pest management in California citrus: history and perspective. Citrograph Mar/Apr: 32-43.

Morse JG, Grafton-Cardwell. 2012b. Management of citrus thrips to reduce the evolution of resistance. Citrograph Mar/Apr: 22-30.

Morse JG, Immaraju JA, Brawner OL. 1988. Citrus thrips: looking to the future. Citrograph 73: 112-115.

Mound LA, Jackman DJ. 1998. Thrips in the economy and ecology of Australia, pp. 472-478 *In* Zalucki MP, Drew RAI, White GG [eds.], Pest Management — Future Challenges, Proceedings of the 6th Australian Applied Entomological Research Conference. University of Queensland, St. Lucia, Australia.

Nauen R, Reckmann U, Thomzik J, Thielert W. 2008. Biological profile of spirotetramat (Movento), a new two-way systemic (ambimobile) insecticide against sucking pest species. Bayer CropScience Journal 61: 245-278.

Navarro C, Pastor MT, Ferragut FJ, García-Mari F. 2008. Trips (Thysanoptera) asociados a parcelas de cítricos en la Comunidad Valenciana: abundancia, evolución estacional y distribución espacial. Bolelín de Sanidad Vegetal de Plagas 34: 53-64.

Navarro-Campos C, Aguilar A, Garcia-Marí F. 2012a. Aggregation pattern, sampling plan, and intervention threshold for *Pezothrips kellyanus* in citrus groves. Entomologia Experimentalis et Applicata 142: 130-139.

Navarro-Campos C, Pekas A, Moraza ML, Garcia-Marí FG, Aguilar A. 2012b. Soil-dwelling predatory mites in citrus: their potential as natural enemies of thrips with special reference *to Pezothrips kellyanus* (Thysanoptera: Thripidae). Biological Control 63: 201-209.

Navarro-Campos C, Pekas A, Aguilar A, Garcia-Marí F. 2013. Factors influencing citrus fruit scarring caused by *Pezothrips kellyanus*. Journal of Pesticide Science 86: 459-467.

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

Purvis S. 2002. Talking Thrips in Citrus. Issue 1. http://www.sardi.sa.gov.au/__data/assets/pdf_file/0003/44922/talking_thrips_1.pdf (last accessed 5 Jan 2014).

Salgado VL. 1998. Studies on the mode of action of spinosad: insect symptoms and physiological correlates. Pesticide Biochemistry and Physiology 60: 91-102.

Sorribas J, Garcia-Marí F. 2010. Comparative efficacy of different combinations of natural enemies for the biological control of California red scale in citrus groves. Biological Control 55: 42-48.

Stevens PS, Steven D, Froud KJ. 1998. Kelly's citrus thrips — a tough customer. The Orchardist 71: 58-61.

Tena A, Soto A, García-Marí F. 2008. Parasitoid complex of black scale *Saissetia oleae* on citrus and olives: parasitoid species composition and seasonal trend. Biocontrol 53: 473-487.

Thompson GD, Dutton R, Sparks TC. 2000. Spinosad — a case study: an example from a natural products discovery program. Pest Management Science 56: 696-702.

Urbaneja A, Pascual-Ruiz S, Pina T, Abad-Moyano R, Vanaclocha P, Monton H, Dembilio O, Castanera P, Jacas JA . 2008. Efficacy of five selected acaricides against *Tetranychus urticae* (Acari: Tetranychidae) and their side effects on relevant natural enemies occurring in citrus orchards. Pest Management Science 64: 834-842.

Urbaneja A, Chueca P, Monton H, Pascual-Ruiz S, Dembilio O, Vanaclocha P, Abad-Moyano R, Pina T, Castañera P. 2009. Chemical alternatives to malathion for controlling *Ceratitis capitata* (Diptera: Tephritidae), and their side effects on natural enemies in Spanish citrus orchards. Journal of Economic Entomology 102: 144-151.

Urbaneja A, Catalán A, Tena A, Jacas JA. 2013. Gestión Integrada de Plagas. http://gipcitricos.ivia.es (last accessed 5 Jan 2014).

Vanaclocha P, Vidal-Quist S, Oeix H, Montón H, Planes L, Catalán J, Tena A, Verdú MJ, Urbaneja A. 2013. Acute toxicity in laboratory tests of fresh and aged residues of pesticides used in citrus on the parasitoid *Aphytis melinus*. Journal of Pest Science 86: 329-333.

Varikou KN, Tsitsipis I, Alexandrakis V. 2010. Effect of diet on development and reproduction of *Pezothrips kellyanus* (Thysanoptera: Thripidae). Annals of the Entomological Society of America 103: 66-70.

Vassiliou VA. 2007. Chemical control of *Pezothrips kellyanus* (Thysanoptera: Thripidae) in citrus plantations in Cyprus. Crop Protection 26: 1579-1584.

Vassiliou VA. 2010. Ecology and behavior of *Pezothrips kellyanus* (Thysanoptera: Thripidae) on citrus. Journal of Economic Entomology 103: 47-53.

Webster KW, Cooper P, Mound LA. 2006. Studies on Kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae): sex attractants, host associations and country of origin. Australian Journal of Entomology 45: 67-74.