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PEST MANAGEMENT

Acute Toxicity of Fresh and Aged Residues of Pesticides to the Parasitoid *Tamarixia radiata* and to the HLB-Bacteria Vector *Diaphorina citri*

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Keywords

Selectivity, IPM, ecotoxicology, sublethal concentration, differential toxicity

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Abstract

One method for controlling the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama, the vector of the putative causal agent of Huanglongbing, uses the parasitoid *Tamarixia radiata* (Waterston). However, the general intensive use of insecticides has reduced the numbers of this parasitoid. This study evaluated the effect of the residual action of 24 insecticides on *T. radiata* and also determined the differential toxicity of insecticides to *D. citri* and *T. radiata*, using three bioassays. In the first, when adults of the parasitoid were exposed to residues of the 24 insecticides, ten were considered short-life (class 1), six slightly persistent (class 2), five moderately persistent (class 3), and three insecticides were considered persistent (class 4), under the IOBC/WPRS classification system. The second bioassay evaluated the sublethal concentrations of the persistent insecticides (formetanate, dimethoate, spinosad). Increasing the concentrations of the insecticides increased the number that were classified as persistent. In the third bioassay, evaluation of the differential toxicity of eight insecticides to the ACP and the parasitoid showed that chlorpyrifos and bifenthrin were more harmful to *T. radiata.* Therefore, these two insecticides are not recommended for application at the time of parasitoid release. Cypermethrin, imidacloprid, and dimethoate caused higher mortality of *D. citri* and are most often recommended in IPM programs. The choice of an insecticide for the control of citrus pests must be made with care, aiming to preserve the natural enemies in the ecosystem, and thereby contribute to the success of biological control.

Introduction

Huanglongbing (HLB) or greening disease is currently the main phytosanitation problem affecting citrus production worldwide (Alemán *et al* 2007, Grafton-Cardwell *et al* 2013). HLB is associated with "*Candidatus* Liberibacter asiaticus" and "*Candidatus* Liberibacter americanus" (Coletta-Filho *et al* 2004, Teixeira *et al* 2005). These phloem-limited bacteria are transmitted by the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Hall *et al* 2013, Yamamoto *et al* 2014). HLB was discovered in São Paulo, Brazil, in 2004 (Teixeira *et al* 2005) and in South Florida, USA, in 2005, and has since been found in Belize, Mexico, Texas, and California (Grafton-Cardwell *et al* 2013). In both Brazil and Florida, the disease has spread rapidly through commercial and residential citrus plantings.

The management of HLB is presently based on the use of healthy nursery trees, elimination of HLB-symptomatic plants, and especially on the control of *D. citri* (Gottwald 2010). Although it is possible to use systemic insecticides, most insecticide applications are made by foliar applications (more than 25 times a year) (Belasque *et al* 2010), which may negatively affect the natural enemies, occasioning outbreaks of secondary pests, resurgence of target pests, and selection of resistant populations (Yamamoto & Bassanezi 2003, Tiwari *et al* 2011, Guedes & Cutler 2013).

Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) is a parasitoid of *D. citri* that has been released as part of biological-control programs for this pest (Pluke *et al* 2008, Qureshi *et al* 2009, Hall & Nguyen 2010, Williams *et al* 2013). It is a specialized ectoparasitoid (Zuparko *et al* 2011) that preferentially develops in third to fifth instar nymphs of *D. citri* (Skelley & Hoy 2004, Hall *et al* 2013) and also feeds on eggs and first to third instar nymphs (Chu & Chien 1991). The combined effects of parasitism and feeding allow a single female of *T. radiata* to eliminate up to 500 psyllid nymphs during its lifetime (Chu & Chien 1991).

In several countries where the disease occurs, successive releases of *T. radiata* have been conducted in areas of HLB management programs, as well as in areas containing the alternative host of *D. citri*, *Murraya paniculata* (L.) Jack (Rutaceae), and in areas near commercial orchards, which are being used for multiplying the ectoparasitoid (Parra *et al* 2010). However, the use of broad-spectrum insecticides may interfere with the biological control exerted by *T. radiata*. In São Paulo state, parasitism rates that were formerly 80% declined to less than 26% due to increased use of pesticides to control *D. citri* (Gomez-Torres *et al* 2006, Parra *et al* 2010, Paiva & Parra 2012).

To prevent this problem, pest management programs should use selective insecticides that cause relatively little harm to the parasitoid. However, only a few studies have evaluated the toxicity of pesticides to *T. radiata*, such as those of Hall and Nguyen (2010), who among 16 pesticides found only four were compatible with the parasitoid; Tiwari and Stelinski (2013) who reported the low toxicity of cyantraniliprole to *T. radiata*; Beloti *et al* (2015) who tested 25 insecticides and found only five selective; and Lira *et al* (2015), who reported only one acaricide considered short lived, among 16 tested. In view of the importance of using the parasitoid *T. radiata* in IPM programs for *D. citri*, this study evaluated the duration of the harmful effects of insecticides that are recommended for the control of insect pests in citrus, on adults of *T. radiata*, in order to contribute to IPM programs that integrate chemical and biological control. The study also compared the toxicity of insecticides to the parasitoid and to *D. citri*.

Material and Methods

Insects

The populations of *T. radiata* and *D. citri* used in the experiments were obtained from the Insect Biology Laboratory and the Integrated Pest Management Laboratory in the Department of Entomology and Acarology of the 'Luiz de Queiroz' College of Agriculture/University of São Paulo (ESALQ/USP) rearing stocks maintained for several generations on seedlings of orange jasmine, *Murraya paniculata* (L.) Jack (Rutaceae).

The parasitoids were reared on seedlings of orange jasmine, *M. paniculata* and using fourth and/or fifth instars *D. citri* nymphs as substrate for female oviposition in acrylic cages (90 × 50 × 50 cm) and in a controlled room (temperature at 25 ± 2°C, relative humidity (RH) 70 ± 10%, and a photoperiod of 14 L:10 D h), as described by Parra *et al* (2016).

Insecticides

We assessed the effects of 24 insecticides that are recommended for integrated citrus production in Brazil, on the parasitoid *T. radiata*. All products were tested at the Highest Field Concentrations (HRFC) recommended by the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) (AGROFIT 2016). The insecticides and concentrations (g a.i. liter^{-1}) used in the bioassays are listed in Table 1. The insecticides were diluted in tap water (pH 7.0–7.5) for all bioassays.

Duration of harmful effects of insecticides on adults of Tamarixia radiata

Five Valencia sweet orange seedlings (*Citrus sinensis* (L.) Osbeck (Rutaceae)), grown in 2-L pots, 80 cm tall, and with 20 mature leaves, for each treatment, were sprayed with the insecticides or tap water (control treatment) until runoff point using a Guarany backpack sprayer equipped with a TXVS-4 conical nozzle. After the treatments were applied, the seedlings were placed in a greenhouse. Three, 7, 10, 17, 24, and 30 days after the spraying, one fully expanded leaf, which was present at the time of spraying, was randomly removed from each plant and leaf discs of 4.0-cm diameter were obtained with the aid of a metallic punch. The discs were placed in Petri dishes (4.5-cm diameter) containing a gelled mixture of water-agar to 2.5% to maintain moisture for the leaf disc; each dish was considered an experimental unit. Later, ten *T. radiata* adults up to 48 h old, of mixed gender from the rearing colony, were anesthetized with CO_2 for 5 s and released in each dish. The dishes were closed with the lid, which had a hole (covered with voile

Table 1 Insecticides used in bioassays either harmful effects or differential toxicity or both, with their concentrations and chemical group.

<table>
<tr><th colspan="2">Insecticide</th><th rowspan="2">Trade name</th><th rowspan="2">Concentration used (g a.i. L^{−1})</th><th rowspan="2">Chemical group</th></tr>
<tr><th>Harmful effects</th><th>Differential toxicity between D. citri and T. radiata</th></tr>
<tr><td>Thiamethoxam</td><td></td><td>Actara® 25 WG</td><td>0.025</td><td>Neonicotinoid</td></tr>
<tr><td colspan="2">Cypermethrin</td><td>Akito®</td><td>0.025</td><td>Pyrethroid</td></tr>
<tr><td>Chlorantraniliprole + lambda-cyhalothrin</td><td></td><td>Ampligo®</td><td>0.02 + 0.01</td><td>Anthranilamide + Pyrethroid</td></tr>
<tr><td>Buprofezin</td><td></td><td>Applaud®</td><td>0.5</td><td>Thiadiazinone</td></tr>
<tr><td>Azadirachtin</td><td></td><td>Azamax®</td><td>0.03</td><td>Tetranortriterpenoide</td></tr>
<tr><td></td><td>Fenpropathrin</td><td>Danimen® 30 EC</td><td>0.150</td><td>Pyrethroid</td></tr>
<tr><td>Deltamethrin</td><td></td><td>Decis Ultra® 10 EC</td><td>0.0075</td><td>Pyrethroid</td></tr>
<tr><td>Formetanate</td><td></td><td>Dicarzol®</td><td>0.15</td><td>Phenyl methylcarbamate</td></tr>
<tr><td colspan="2">Lambda-cyhalothrin + thiamethoxam</td><td>Engeo Pleno®</td><td>0.02 + 0.02</td><td>Pyrethroid + neonicotinoid</td></tr>
<tr><td>Imidacloprid</td><td></td><td>Evidence® 70 WG</td><td>0.03</td><td>Neonicotinoid</td></tr>
<tr><td>Phosmet</td><td></td><td>Imidan® 50 WP</td><td>0.25</td><td>Organophosphate</td></tr>
<tr><td colspan="2">Lambda-cyhalothrin</td><td>Karate Zeon® 5 CS</td><td>0.01</td><td>Pyrethroid</td></tr>
<tr><td colspan="2">Chlorpyrifos</td><td>Lorsban® 48 BR</td><td>0.72</td><td>Organophosphate</td></tr>
<tr><td>Malathion</td><td></td><td>Malathion® 100 EC</td><td>1.5</td><td>Organophosphate</td></tr>
<tr><td>Tebufenozide</td><td></td><td>Mimic® 24 SC</td><td>0.12</td><td>Diacylhydrazine</td></tr>
<tr><td>Mineral Oil</td><td></td><td>Mineral Oil Argenfrut®</td><td>8.46</td><td>Aliphatic hydrocarbon</td></tr>
<tr><td>Vegetable Oil</td><td></td><td>Vegetable Oil Nortox®</td><td>9.30</td><td>Fatty acid esters</td></tr>
<tr><td colspan="2">Dimethoate</td><td>Perfekthion®</td><td>0.8</td><td>Organophosphate</td></tr>
<tr><td colspan="2">Imidacloprid</td><td>Provado® 20 SC</td><td>0.04</td><td>Neonicotinoid</td></tr>
<tr><td>Acetamiprid</td><td></td><td>Saurus®</td><td>0.06</td><td>Neonicotinoid</td></tr>
<tr><td>Esfenvalerate</td><td></td><td>Sumidan® 15 SC</td><td>0.02</td><td>Pyrethroid</td></tr>
<tr><td></td><td>Bifenthrin</td><td>Talstar® 10 EC</td><td>0.02</td><td>Pyrethroid</td></tr>
<tr><td>Pyriproxyfen</td><td></td><td>Tiger®</td><td>0.006</td><td>Ether pyridine</td></tr>
<tr><td>Spinosad</td><td></td><td>Tracer®</td><td>0.07</td><td>Spinosyns</td></tr>
<tr><td>Etofenprox</td><td></td><td>Trebon® 10 SC</td><td>0.025</td><td>Pyrethroid ether</td></tr>
<tr><td>Beta-cyfluthrin</td><td></td><td>Turbo®</td><td>0.012</td><td>Pyrethroid</td></tr>
</table>

fabric) in the center to allow gas exchange. A drop of pure honey (~1 mm^3) was placed once a week on the voile to serve as food for the parasitoids during the evaluation period. Five replicates were used for each treatment ($n = 50$). The bioassay was conducted in a controlled room (temperature at 25 ± 2°C, RH 70 ± 10%, and a photoperiod of 14 L:10 D h), using a completely randomized design.

The mortality of the parasitoids was assessed 24 h after they were exposed to the treated leaves. Insects unable to move when prodded with a fine brush were considered dead. The mortality data for each insecticide and assessment period were corrected by the formula of Abbott (1925). The insecticides that caused less than 30% of mortality compared to the control treatment (tap water) were classified according to the persistence scale proposed by the International Organization for Biological and Integrated Control of Noxious Animals and Plants/West Palearctic Regional Section (IOBC/WPRS): class 1—short-life (< 5 days), class 2—slightly persistent (5–15 days), class 3—moderately persistent (16–30 days), and class 4—persistent (> 30 days) (Van de Veire *et al* 2002).

Duration of harmful effects of different concentrations of insecticide on adults of Tamarixia radiata

To investigate the effects of different concentrations of insecticides for adults of *T. radiata*, dimethoate, formetanate, and spinosad, the insecticides classified as persistent (class 4) in the bioassay for duration of harmful effects were selected. For each insecticide, the HRFC and 0.75×, 0.50×, and 0.25× HRFC were used (Table 2).

The methods and conditions were the same as in the first bioassay (duration of harmful effects of insecticides on adults of *T. radiata*).

Differential toxicity of insecticides to Diaphorina citri and Tamarixia radiata

To assess the differential toxicity to the Asian citrus psyllid *D. citri* and the parasitoid *T. radiata*, the insecticides that are most often used in citrus were selected (Table 1). Adults of *D. citri* and *T. radiata* up to 48 h old, of mixed gender and from the rearing colony, were exposed to insecticide residues sprayed on the Valencia sweet orange seedlings, following the same bioassay method (duration of harmful effects of insecticides on adults of *T. radiata*). Five replicates with ten adults each were used for each treatment ($n = 50$). The bioassay was conducted in a controlled room (temperature 25 ± 2°C, RH 70 ± 10%, and a photoperiod of 14 L:10 D h), using a completely randomized design.

Quasi-binomial models (McCullagh & Nelder 1989) were fitted to the mortality data for each insecticide, with a different linear predictor for each species. Submodels (parallel and coincident linear predictors) were also fitted and compared using *F* tests. Goodness-of-fit was assessed using half-normal plots with simulated envelopes (Demétrio *et al* 2014). All analyses were carried out using software R (R Core Team 2017).

Results

Duration of harmful effects of insecticides on adults of Tamarixia radiata

The duration of the harmful effects of the insecticides on adults of *T. radiata*, sprayed on citrus seedlings, showed different IOBC classes, which are present in Table 3. Formetanate, dimethoate, and spinosad were considered persistent for the parasitoid (> 30 days, class 4) according to the IOBC/WPRS criteria (Table 3). Thiamethoxam, cypermethrin, deltamethrin, phosmet, and etofenprox were considered moderately persistent (16–30 days, class 3) (Table 3). Buprofezin, lambda-cyhalothrin + thiamethoxam, lambda-cyhalothrin, malathion, imidacloprid (0.04), and esfenvalerate were considered slightly persistent (5–15 days, class 2) (Table 3). Ten of the insecticides tested were harmless to the parasitoid, being considered as short-life (< 5 days) (class 1): chlorantraniliprole + lambda-cyhalothrin, azadirachtin, imidacloprid (0.03), chlorpyrifos, tebufenozide, mineral oil, vegetable oil, acetamiprid, pyriproxyfen, and beta-cyfluthrin (Table 3).

Duration of harmful effects of different concentrations on adults of Tamarixia radiata

Evaluation of different concentrations of the insecticides considered persistent showed that increasing the concentration of spinosad increased the duration of the harmful effect (persistence) (Fig 1). With 0.25× HRFC, the insecticide was considered slightly persistent (class 2); with 0.50× and 0.75× HRFC, the insecticide was considered moderately persistent (class 3); and with the HRFC, the insecticide was considered persistent (class 4).

Table 2 Insecticides and their different concentrations used in bioassays of sublethal concentrations on adults of *Tamarixia radiata.*

Treatment	HRFC	0.75× HRFC	0.50× HRFC	0.25× HRFC
Spinosad	0.070	0.0525	0.035	0.0175
Formetanate	0.150	0.1125	0.075	0.0375
Dimethoate	0.800	0.600	0.400	0.200

HRFC Highest Recommended Field Concentration by MAPA.

Formetanate caused high mortality of the parasitoids, even if only 0.25× HRFC was used (Fig 1), and was classified as moderately persistent with this concentration (class 3). Formetanate was classified as persistent (class 4) at the other sublethal concentrations tested.

Dimethoate, in contrast to the results from the persistence test, was classified as slightly persistent in all concentrations tested.

Differential toxicity of insecticides to Diaphorina citri and Tamarixia radiata

The effects of the insecticides on *D. citri* and *T. radiata* differed significantly (Table 4). A significantly different vs. parallel test means that the mortality curves of two species differ. When the same test is not significant, but the parallel vs. coincident test is significant, this means that the mortality curves are parallel, i.e., the mortality decreases similarly over time for both species, but the mortality of one species is always higher than that of the other.

The mortality curves for cypermethrin, dimethoate, and imidacloprid (0.04) were parallel when the insects were compared; but over time, *D. citri* showed higher mortality than *T. radiata* (Fig 2). However, dimethoate caused 100% mortality of the parasitoid at 3 days after spraying (DAS).

Lambda-cyhalothrin + thiamethoxam, chlorpyrifos, and bifenthrin also showed parallel mortality curves. However, over time, the mortality of *T. radiata* exceeded that of *D. citri* (Fig 2).

Fenpropathrin caused higher mortality of *D. citri* after 3 DAS; but after 10 DAS, the mortality of the parasitoid was higher than the psyllid. The mortality caused by lambda-cyhalothrin was similar for both species, but presenting a more pronounced mortality on the parasitoid (Fig 2).

Table 3 Mortality ± SE (%) of *T. radiata* adults 1, 3, 7, 10, 17, 24, and 30 days after spraying of the insecticides, and the IOBC/WPRS classes.

Treatment	Concentration used (g a.i. L^{-1})	Mortality ± SE (%)—days after spraying (DAS)						IOBC/WPRS class[a]
		3	7	10	17	24	30	
Control	–	0.0 ± 0.0	2.0 ± 2.0	4.0 ± 2.4	2.0 ± 2.0	2.0 ± 2.0	2.0 ± 2.0	–
Acetamiprid	0.06	36.0 ± 16.0	8.0 ± 4.0	8.0 ± 2.0	10.0 ± 4.0	10.0 ± 6.0	16.0 ± 10.0	1
Azadirachtin	0.03	8.0 ± 2.0	2.0 ± 2.0	4.0 ± 2.4	4.0 ± 2.4	2.0 ± 2.0	4.0 ± 2.4	1
Beta-cyfluthrin	0.012	44.0 ± 14.0	8.0 ± 6.0	24.0 ± 7.0	28.0 ± 5.0	24.0 ± 9.0	6.0 ± 4.0	1
Buprofezin	0.5	82.0 ± 7.0	40.0 ± 13.0	18.0 ± 13.0	28.0 ± 7.0	18.0 ± 6.0	32.0 ± 15.0	2
Chlorantraniliprole + lambda-cyhalothrin	0.02 + 0.01	38.0 ± 3.7	16.0 ± 9.3	8.0 ± 2.0	2.0 ± 2.0	2.0 ± 2.0	4.0 ± 2.4	1
Chlorpyrifos	0.72	46.0 ± 2.4	22.0 ± 2.0	30.0 ± 3.2	8.0 ± 4.9	8.0 ± 2.0	6.0 ± 2.4	1
Cypermethrin	0.025	96.0 ± 2.0	68.0 ± 16.0	36.0 ± 13.0	30.0 ± 7.0	14.0 ± 9.0	14.0 ± 4.0	3
Deltamethrin	0.0075	78.0 ± 12.0	32.0 ± 17.0	34.0 ± 13.0	30.0 ± 9.0	26.0 ± 7.0	24.0 ± 13.0	3
Dimethoate	0.8	100.0 ± 0.0	90.0 ± 6.0	96.0 ± 4.0	30.0 ± 12.0	34.0 ± 12.0	42.0 ± 10.0	4
Esfenvalerate	0.02	48.0 ± 2.0	28.0 ± 2.0	38.0 ± 2.0	24.0 ± 5.1	18.0 ± 5.8	12.0 ± 4.9	2
Etofenprox	0.025	96.0 ± 4.0	66.0 ± 12.0	80.0 ± 6.0	58.0 ± 11.0	20.0 ± 13.0	12.0 ± 7.0	3
Formetanate	0.15	92.0 ± 6.0	96.0 ± 4.0	100.0 ± 0.0	68.0 ± 10.0	44.0 ± 16.0	46.0 ± 14.0	4
Imidacloprid	0.03	30.0 ± 4.5	20.0 ± 4.5	22.0 ± 6.6	26.0 ± 2.4	22.0 ± 3.7	4.0 ± 2.4	1
Imidacloprid	0.04	40.0 ± 3.2	36.0 ± 6.8	24.0 ± 6.8	8.0 ± 5.8	14.0 ± 2.4	8.0 ± 2.0	2
Lambda-cyhalothrin	0.01	70.0 ± 10.0	40.0 ± 14.0	32.0 ± 18.0	24.0 ± 12.0	20.0 ± 5.0	2.4 ± 1.3	2
Lambda-cyhalothrin + thiamethoxam	0.02 + 0.02	50.0 ± 0.0	48.0 ± 2.0	30.0 ± 3.2	20.0 ± 3.2	16.0 ± 4.0	16.0 ± 4.0	2
Malathion	1.5	44.0 ± 0.40	34.0 ± 0.40	16.0 ± 4.0	14.0 ± 2.4	6.0 ± 4.0	4.0 ± 2.4	2
Mineral oil	8.46	12.0 ± 4.0	6.0 ± 2.0	12.0 ± 2.0	18.0 ± 4.0	8.0 ± 6.0	8.0 ± 4.0	1
Phosmet	0.25	40.0 ± 4.5	44.0 ± 4.0	44.0 ± 2.4	34.0 ± 6.0	22.0 ± 3.7	6.0 ± 4.0	3
Pyriproxyfen	0.006	46.0 ± 2.4	18.0 ± 3.7	8.0 ± 3.7	4.0 ± 2.4	6.0 ± 2.4	6.0 ± 2.4	1
Spinosad	0.07	100.0 ± 0.0	80.0 ± 15.0	98.0 ± 2.0	84.0 ± 7.0	48.0 ± 15.0	56.0 ± 10.0	4
Tebufenozide	0.12	28.0 ± 12.0	10.0 ± 5.0	16.0 ± 6.0	30.0 ± 14.0	18.0 ± 7.0	12.0 ± 8.0	1
Thiamethoxam	0.025	48.0 ± 2.0	40.0 ± 3.2	42.0 ± 5.8	38.0 ± 2.0	18.0 ± 2.0	4.0 ± 2.4	3
Vegetable oil	9.30	34.0 ± 16.0	6.0 ± 2.0	10.0 ± 4.0	16.0 ± 10.0	10.0 ± 5.0	18.0 ± 9.0	1

[a] IOBC/WPRS class based on the duration of the harmful effect of insecticides: class 1 = short-life (< 5 days), class 2 = slightly persistent (5–15 days), class 3 = moderately persistent (16–30 days), and class 4 = persistent (> 30 days).

Discussion

In our study, the interaction complexes between the insecticides and the plant were not analyzed. On the other hand, the effects of the residues of 24 insecticides recommended for citrus were evaluated on the ectoparasitoid *T. radiata.* The results showed that chlorantraniliprole + lambda-cyhalothrin, azadirachtin, imidacloprid (0.03), chlorpyrifos, tebufenozide, mineral oil, vegetable oil, acetamiprid, pyriproxyfen, and beta-cyfluthrin were considered as short-life.

For azadirachtin and tebufenozide, our results agree with those reported by Biondi *et al* (2013), Momanyi *et al* (2012), and Zhao *et al* (2012), who classified these insecticides as short-life (class 1) for different species of parasitoids. Tebufenozide, a member of the insect growth-regulator group (IGRs), affects larvae more than adults (Sieber and Rembold 1983), and together with azadirachtin, a botanical product, is generally considered safe for the environment and for natural enemies (Chen *et al* 2013) and can be recommended for IPM programs.

For *T. radiata*, buprofezin was slightly persistent. This result agrees with the findings reported by Rugno *et al* (2016) for the lacewing *Ceraeochrysa cincta* (Schneider) (Neuroptera: Chrysopidae). This insecticide is a chitin biosynthesis inhibitor type 1 (IGR) and affects immature stages more than adults.

The three neonicotinoids studied differed in their effects. Acetamiprid showed the lowest persistence of harmful effects, followed by imidacloprid (class 1 or 2 depending on the concentration) and thiamethoxam. This difference may be either related to the generation of neonicotinoids or due to molecules degradation. Thiamethoxam is a second-generation neonicotinoid (Maienfisch *et al* 2001) that acts differently from the first-generation imidacloprid and acetamiprid. Thiamethoxam is able to bind to mixed nicotinic/muscarinic receptors (Lapied *et al* 1990), and this mechanism

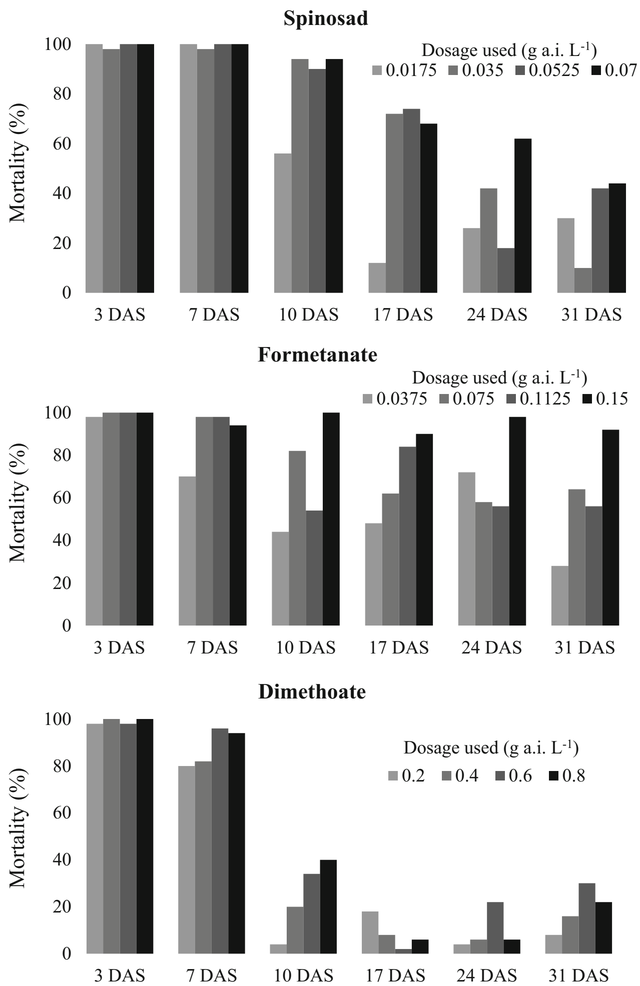

Fig 1 Mortality (%) of *T. radiata* adults 1, 3, 7, 10, 17, 24, and 30 days after spraying of the different concentrations of three insecticides.

or site of action may increase the effect of thiamethoxam on *T. radiata*, unlike imidacloprid and acetamiprid.

The divergent results for the action on *T. radiata* probably are not related to the type of pyrethroid. With the exception of etofenprox, which is a non-ester pyrethroid, the others are type II (Soderlund & Bloomquist 1989). The difference between the results obtained here may be related to the pyrethroid generation. Deltamethrin and cypermethrin, the oldest pyrethroids (Khambay 2002), were more harmful to the parasitoid, while the newer pyrethroids were more selective and less persistent.

The results for lambda-cyhalothrin and malathion differ from those obtained by Momanyi *et al* (2012), who classified lambda-cyhalothrin as persistent (class 4) and malathion as moderately persistent (class 3) for two species of *Trichogramma*. These differences in classification can be explained by the difference in the substrate, by the concentrations and inert components of the insecticides, and also by the insect species used in the bioassays. Species of the genus *Trichogramma* can be more sensitive than adults of *T. radiata*, perhaps because of their smaller body size. While adult males of *Trichogramma* measure 0.5 to 0.65 mm in length and adult females from 0.46 to 0.62 mm (Carver 1978), the body length of *T. radiata* adults ranges from 0.92 to 1.3 mm (Waterston 1922, Onagbola *et al* 2009, Chen & Stansly 2014).

In our study, formetanate was classified as persistent (class 4). These results differ from those obtained by Brunner *et al* (2001), who found that formetanate caused high mortality in adults of *Colpoclypeus florus* (Walker) (Hymenoptera: Eulophidae) up to 14 DAS. This divergence may be related to differences in the commercial product used, i.e., the concentrations and inert components of the insecticides.

Spinosad is sometimes classified as reduced-risk environmentally and toxicologically, considered an organic material, and usually less harmful to predators, but wasp parasitoids are significantly more susceptible to its effects (Williams *et al* 2003). In our study, spinosad was classified as persistent (class 4) for *T. radiata* adults. Similarly, Biondi *et al* (2013) classified spinosad as harmful to *B. nigricans*. According to Biondi *et al* (2012), spinosad is acutely toxic to many hymenopteran parasitoids, with high levels of mortality, marked typical poisoning symptoms, and reduced mobility after exposure.

Therefore, insecticides such as formetanate, dimethoate, and spinosad should be used judiciously in IPM programs,

Table 4 Statistical tests for nested models fitted to the data for differential toxicity per species ($P < 0.05$).

Insecticide	Different vs. parallel linear predictors	Parallel vs. coincident linear predictors
Cypermethrin	$F_{1,56} = 0.02$, $P = 0.88$	$F_{1,57} = 29.41$, $P < 0.01$
Fenpropathrin	$F_{1,56} = 7.62$, $P < 0.01$	$F_{1,57} = 27.05$, $P < 0.01$
Lambda-cyhalothrin + thiamethoxam	$F_{1,56} = 2.59$, $P = 0.11$	$F_{1,57} = 5.50$, $P = 0.02$
Lambda-cyhalothrin	$F_{1,56} = 0.42$, $P = 0.52$	$F_{1,57} = 0.01$, $P = 0.91$
Chlorpyrifos	$F_{1,56} = 0.24$, $P = 0.65$	$F_{1,57} = 55.11$, $P < 0.01$
Dimethoate	$F_{1,56} = 0.48$, $P = 0.49$	$F_{1,57} = 19.93$, $P < 0.01$
Imidacloprid	$F_{1,56} = 2.57$, $P = 0.11$	$F_{1,57} = 6.22$, $P = 0.02$
Bifenthrin	$F_{1,56} = 0.06$, $P = 0.81$	$F_{1,57} = 10.78$, $P < 0.01$

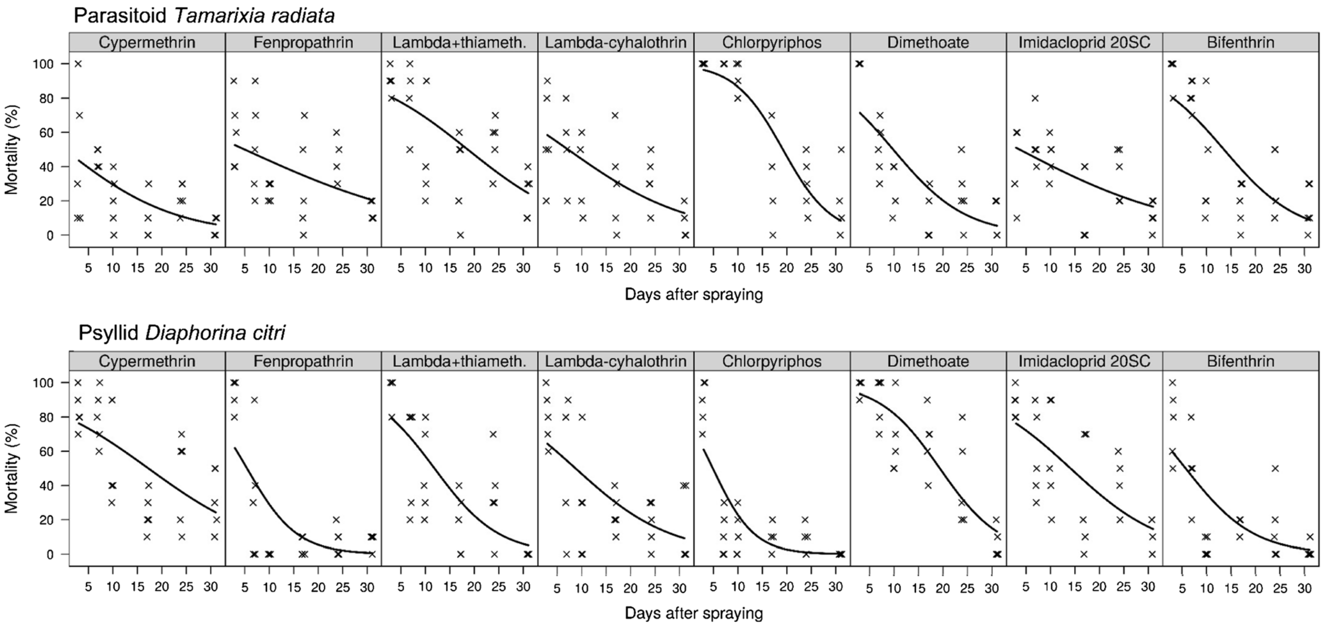

Fig 2 Mortality (%) of the parasitoid *Tamarixia radiata* and the psyllid *Diaphorina citri* caused by the most frequently used insecticides over time.

during periods when *T. radiata* does not occur naturally in the field or that do not coincide with parasitoid releases.

Similar studies of persistence should be performed under field conditions to determine the most appropriate time to release *T. radiata* after a particular insecticide is sprayed. Plants in the field are exposed to rain, moisture, sunlight, and other abiotic factors that can affect the persistence of insecticides.

Evaluation of the differential toxicity of insecticides to the psyllid *D. citri* and the parasitoid *T. radiata* showed that lambda-cyhalothrin and lambda-cyhalothrin + thiamethoxam affected the two species similarly. Field studies have found that these insecticides reduced the psyllid population up to 33 DAS (Yamamoto *et al* 2009).

In our study, dimethoate caused high mortality of the psyllid. Similarly, Yamamoto *et al* (2009), evaluating the efficiency of foliar application of dimethoate, observed reduction of the adult population within 1 h after spraying, and the population remained low until 33 DAS.

Cypermethrin caused higher mortality of *D. citri* than of *T. radiata* during the entire bioassay and can be recommended for use in IPM programs. Imidacloprid (0.04), despite causing high mortality of the psyllid at 3 DAS, did not differ in the other evaluation periods for both insects. When applied as a soil drench, this insecticide is effective against *D. citri* up to 30 DAS (Miranda *et al* 2016).

Chlorpyrifos and bifenthrin were more harmful to the parasitoid than to the psyllid. Therefore, these insecticides should be avoided during periods when the parasitoids are released or when they occur naturally in the field.

Insecticides of the same chemical group affected the mortality of the pest and the natural enemy differently. While the organophosphate dimethoate caused higher mortality of the psyllid, chlorpyrifos caused higher mortality of the parasitoid. Among the pyrethroids, cypermethrin caused high mortality of the psyllid, fenpropathrin caused similar mortalities of both insects, and bifenthrin caused high mortality of the parasitoid. These variations in mortality can be aggravated or attenuated because of the active ingredients, inert substances, formulations, toxicological classification, molecular weight, application method, weather conditions, and different concentrations of the insecticides, which may raise or lower the relative mortality of different insect species (Cox & Surgan 2006).

The few studies reporting the differential toxicity of insecticides to *D. citri* and *T. radiata* indicate that, when the mortality of the psyllid was higher than that of the parasitoid, the difference in the toxicity to the two species was related to physiological selectivity (Ripper *et al* 1951, Degrande *et al* 2002). That is, when these insects came into direct contact with the insecticide residues, the insecticide impacted the pest more than the natural enemy (Degrande *et al* 2002, Foerster 2002). Physiological selectivity involves the processes of absorption, penetration, transport, and activation of insecticides, which, when they act at different intensities, result in different toxicity to two species (Foerster 2002).

The information provided by this study, and knowledge of the lethal and sublethal effects of different pesticides on the parasitoid *T. radiata* (Beloti *et al* 2015, Lira *et al* 2015), will enable citrus growers to select the most appropriate insecticides that both control the psyllid *D. citri* and are less toxic to its parasitoid *T. radiata*. This will allow both chemical and biological-control methods to be used in conjunction, in accordance with one of the IPM principles.

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