


Susceptibility of *Euseius concordis* (Mesostigmata: Phytoseiidae) to pesticides used in citrus production systems

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Abstract *Euseius concordis* (Chant) is an important predatory mite found in citrus orchards. The toxicity of 19 pesticides used in citrus orchards on biological and population parameters of this mite was assessed. Our results indicated that formetanate hydrochloride, dimethoate and phosmet were highly harmful (100% mortality) to *E. concordis*. Carbosulfan, diflubenzuron, fenpropathrin, gamma-cyhalothrin, imidacloprid, lambda-cyhalothrin, lambda-cyhalothrin + thiamethoxam, mineral and vegetable oils, spinosad and thiamethoxam reduced the female's survival and/or fecundity, and were moderately harmful to *E. concordis*. Besides the acute toxicity, carbosulfan and formetanate hydrochloride were highly persistent [>30 days after spraying (DAS)]; dimethoate was moderately persistent (16–30 DAS); spinosad, gamma-cyhalothrin, lambda-cyhalothrin and lambda-cyhalothrin + thiamethoxam were slightly persistent (5–15 DAS); and the other pesticides were considered to be short-lived (<5 DAS). All compounds except lambda-cyhalothrin and thiamethoxam increased the pre-oviposition period in the female offspring. Carbosulfan, deltamethrin, diflubenzuron, etofenprox, fenpropathrin, gamma-cyhalothrin, mineral and vegetable oils, pyriproxyfen and tebufenozide reduced offspring fecundity, whereas thiamethoxam increased the fecundity. Mineral and vegetable oils reduced female longevity of the predator mite. Regarding population effects, imidacloprid, lambda-cyhalothrin, lambda-cyhalothrin + thiamethoxam and thiamethoxam led to an increase in net reproductive rate (R_o), intrinsic rate of increase (r), and finite rate of increase (λ) of *E. concordis*. Diflubenzuron, etofenprox, and mineral and vegetable oils reduced R_o , r and λ . All pesticides except beta-cypermethrin, fenpropathrin and imidacloprid reduced the mean generation time (T) of the predator. Therefore, semi-field and field studies are needed to assess the compatibility of these compounds with *E. concordis* before adoption in IPM programs.

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Introduction

The biological control of crop pests by predators and parasitoids is a main component of integrated pest management (IPM), which aims to reduce pest populations below the level of economic damage (Zappalà et al. 2013; Maoz et al. 2014). Predatory mites are important biological control agents in agroecosystems because they reduce the population levels of pest mites, which cause direct damage or act as pathogen vectors that limit the normal growth, development and reproduction of the plants. Furthermore, conservation and/or augmentation of these predators can reduce the amount of pesticides used in production systems, decreasing production costs and environmental contamination (Sato 2005). Phytoseiids are the most common and abundant predatory mites found in citrus orchards in Brazil (Matioli and Oliveira 2007). In a survey by Silva et al. (2012) in São Paulo state, *Euseius concordis* (Chant) (Mesostigmata: Phytoseiidae) was most abundant, comprising 98.3% of the phytoseiids found in citrus orchards. These authors also observed a negative relationship between the predator population level and the population level of three citrus pests, *Panonychus citri* (McGregor) (Prostigmata: Tetranychidae), *Brevipalpus* spp. Donnadieu (Prostigmata: Tenuipalpidae) and *Phyllocoptruta oleivora* (Ashmead) (Prostigmata: Eriophyidae). This predator mite can also feed on pollen and plant exudates (Croft et al. 2004; Moraes and Flechtman 2008), which contributes to its survival and reproduction in periods of low prey availability. The use of these alternative foods can favor the maintenance and multiplication of the mite under laboratory conditions for inundative or inoculative releases in production areas (Albuquerque and Moraes 2008).

Although *E. concordis* has good potential as a biological control agent, the use of pesticides for managing pests such as *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), vector of the bacteria ‘*Candidatus Liberibacter* spp.’ associated with huanglongbing (HLB), may reduce the population levels and effectiveness of this predatory mite in citrus orchards. Overuse of pesticides can also induce a resurgence of the target pest, outbreaks of secondary pests, and selection of populations that are resistant (Biondi et al. 2012; Guedes and Cutler 2014; Zhan et al. 2015; Guedes et al. 2016), which increases production costs and reduces the efficacy of the technique and the environmental sustainability of a system.

In addition to causing mortality, pesticides can also affect the development of immatures, fecundity, fertility, longevity and sex ratio, and decrease the mites’ predation potential, mobility, orientation and feeding activity (Desneux et al. 2007; Tuelher et al. 2014; Guedes et al. 2016), and reduce their biocontrol services (Biondi et al. 2015). Several studies have demonstrated acute toxicity and sublethal effects of pesticides on predatory mites (Reis et al. 1998; Castagnoli et al. 2005; Silva and Oliveira 2006; Poletti et al. 2008; Pozzebon et al. 2010, 2015; Beers and Schmidt 2014), but few studies have considered biological and population parameters in assessing the impacts of pesticides on *E. concordis* (Costa et al. 2014). Population parameters are a useful tool for analyzing the effects of pesticides on predators and parasitoids (Biondi et al. 2015), because they reflect the cumulative effects on the survival, development and reproduction of natural enemies (Southwood and Henderson 2000). These studies also contribute to understanding the changes in density and population dynamics of predatory mites after pesticide applications (Zanardi et al. 2017).

In this study, we assessed the lethal and sublethal effects on *E. concordis* of 19 pesticides used in citrus orchards. We also determined the duration of the harmful activity of these compounds and estimated the biological and population parameters of the offspring (F1 generation) of this predatory mite. These findings will help understand the impacts of these compounds on *E. concordis*, and they will help in the adoption of management strategies to exploit the action of this biological control agent in citrus production systems.

Materials and methods

Mites

The *E. concordis* colony was established from specimens provided from a rearing colony kept under laboratory conditions and without exposure to pesticides for the last 5 years. The mites were reared on leaves of copperleaf, *Acalypha wilkesiana* Muell. (Euphorbiaceae), placed with the abaxial side up on a foam layer moistened with deionized water, in plastic trays (38.5 cm long \times 24.5 cm wide \times 6.0 cm high) as described by Zanardi et al. (2017). Moistened cotton-wool strips were used on the edges of the leaves to maintain their turgor and prevent the mites from escaping. On each acalypha leaf, a piece of cotton yarn was placed to provide a shelter and oviposition site for the mites. As food, pollen from narrow-leaved cattail, *Typha angustifolia* L. (Typhaceae), was provided on glass cover slips measuring 2 \times 2 cm, which were replaced every 48 h. The rearing trays were kept in a climate-controlled room at 25 ± 2 °C, $60 \pm 10\%$ relative humidity (RH), and 14 L:10 D h photoperiod.

Chemicals

Nineteen commercial pesticides were tested at the label rates (Agrofit 2016). The active ingredients, trade name, chemical groups, pesticide action mode, and concentrations (mg a.i. L⁻¹) used are given in Table 1.

Bioassays

Bioassays were carried out in a climate-controlled room at 25 ± 2 °C, $60 \pm 10\%$ RH and 14 L:10 D h photoperiod. During the assessment period, fresh cattail pollen (*T. angustifolia*) on glass slides 0.5 \times 0.5 cm was used as mite food. All bioassays were carried out following a fully randomized design.

Effects on adult females (Experiment 1)

Leaves of Valencia sweet orange, *Citrus sinensis* (L.) Osbeck (Rutaceae) were sprayed with 2 mL of solution in a Potter tower (Burkard Scientific, Uxbridge, UK), adjusted to a pressure of 68 kPa, resulting in a deposit of 1.8 ± 0.1 mg cm⁻² of fresh residues, which is consistent with the criteria established for pesticide toxicology studies on natural enemies (Hassan et al. 1994). Deionized water (solvent used for dilution/solubilization of pesticides) was used as a control treatment when the mortality rate did not exceed 10%. After the treatments were sprayed, the leaves were maintained for 3 h in a climate-controlled room to allow the residue to dry. After this period, the experimental units (4.0 cm

Table 1 Pesticides used in bioassays

Active ingredient	Trade name	Chemical group	Manufacturer	Concentration used (mg a.i. L ⁻¹)
Beta-cypermethrin	Akito 10 EC	Pyrethroid	Arysta	30
Carbosulfan	Marshal Star 40 EC	Carbamate	FMC	200
Formetanate hydrochloride	Dicarzol 50 SP	Carbamate	Cross Link	145.5
Deltamethrin	Decis Ultra 10 EC	Pyrethroid	Bayer	7.5
Diflubenzuron	Micromite 24 SC	Benzoylurea	Chemtura	120
Dimethoate	Perfekthion 40 EC	Organophosphate	Basf	800
Etofenprox	Trebon 10 SC	Pyrethroid	Sipcam Agro	2.5
Fenpropathrin	Danimen 30 EC	Pyrethroid	Iharabras	150
Gamma-cyhalothrin	Nexide 15 CS	Pyrethroid	Cheminova	7.5
Imidacloprid	Provado 20 SC	Neonicotinoid	Bayer	100
Lambda-cyhalothrin	Karate Zeon 5 CS	Pyrethroid	Syngenta	10
Lambda-cyhalothrin + thiamethoxam	Engeo Pleno SC	Pyrethroid + Neonicotinoid	Syngenta	26.6 + 35.2
Mineral oil	OPPA BR EC	Oil	Petrobras	12,000
Phosmet	Imidan 50 WP	Organophosphate	Cross Link	750
Pyriproxyfen	Tiger 10 CE	Pyriproxyfen	Sumitomo	100
Spinosad	Tracer 48 SC	Spinosyn	Dow	72
Tebufenozide	Mimic 24 SC	Diacylhydrazine	Dow	120
Thiamethoxam	Actara 25 WG	Neonicotinoid	Syngenta	50
Vegetable oil	Óleo Vegetal Nortox	Oil	Nortox	18,600

diameter) were composed, with Valencia sweet-orange leaves placed on a layer of foam (ca. 1 cm) moistened with deionized water in plastic trays (38.5 × 24.5 × 6.0 cm), and kept in a climate-controlled room. Then, 10 adult females (24–72 h old) were released in each experimental unit. For each treatment, five replicates were used. The mortality of these females was assessed every 24 h for 8 days. Mites that did not react when touched with a fine brush were considered dead. During the assessments, the eggs laid by the females (fecundity) in the experimental units were counted and used to assess the female fertility (number of hatched larvae). For this purpose, the eggs were removed from the experimental units and placed in Petri dishes (3.5 cm diameter, 0.7 cm high) and kept in a climate-controlled room. The number of hatched larvae in each Petri dish was recorded every 24 h for 4 days after oviposition. Eggs that did not generate larvae were considered dead.

Based on the data for mortality and female fecundity and fertility, a reduction coefficient (E) was calculated for each pesticide, using the formula proposed by Bakker et al. (1992):

$$E (\%) = 100 - (100 - M_c) \times E_r,$$

where M_c = total mortality of the immature stage corrected by the formula of Abbott (1925), and E_r = effect on reproduction calculated as $R_1 \times R_2$, where R_1 = ratio between the number of eggs laid by females that developed on the pesticide residues versus the

control, and R_2 = ratio between the number of hatched larvae from females developed on the pesticides residues versus the control. Pesticides were classified according to the standards established by the IOBC/WPRS for extended laboratory studies on natural enemies: 1: harmless ($E < 25\%$), 2: slightly harmful ($25\% \leq E < 50\%$), 3: moderately harmful ($50\% \leq E < 75\%$), and 4: highly harmful ($E \geq 75\%$) (Hassan 1992).

Duration of the harmful effects (Experiment 2)

The duration of harmful effects was assessed for the pesticides that presented a reduction coefficient of $>50\%$ in the acute toxicity bioassay (Experiment 1). For this purpose, seedlings of Valencia sweet orange grown in 12-L pots were sprayed with a volume corresponding to 100 mL m^{-3} canopy, resulting in deposition of ca. 1.8 mg cm^{-2} of fresh residues on the leaf surface, using a manual sprayer model Jacto PJH (Jacto do Brasil, Pompeia, São Paulo, SP, Brazil) equipped with a FL-5VS nozzle cone (Teejet Technologies, São Paulo, SP, Brazil). For each treatment, five seedlings were used. After 3, 7, 10, 17, 24 and 31 DAS, a randomly selected leaf was removed from each seedling and taken to the laboratory to prepare the experimental units, as described in Experiment 1. Then, 10 females (<24 h old) were released into each experimental unit. For each treatment and assessment date, five replicates were used. The female mortality was recorded 24 h after the mites were released in the experimental units. When the pesticides reduced the mite mortality by $<25\%$, the compounds were classified according to a scale of persistence proposed by the IOBC/WPRS: 1: short-life (<5 DAS), 2: slightly persistent (5–15 DAS), 3: moderately persistent (16–30 DAS), and 4: persistent (>30 DAS) (Hassan 1992).

Progeny effects (Experiment 3)

The pesticide effects were assessed for all the compounds, except for formetanate hydrochloride, dimethoate and phosmet, because they caused 100% female mortality (Experiment 1). For this purpose, 12 eggs from each surviving female were collected and transferred to new experimental units made with untreated Valencia sweet-orange leaves, as in the procedure described in Experiment 1. For each treatment, five replicates were used. The survival and duration of the immature stages (larvae, protonymphs and deutonymphs) were assessed every 12 h until the adults emerged. The adults were separated by gender, paired into couples and transferred to new experimental units (without residues) in order to make daily assessments of the pre-oviposition and oviposition periods, fecundity (number of eggs laid by females), fertility (number of hatched larvae), and female and male longevities. When the males died before the females, new males from the laboratory colony were transferred to the experimental units. However, the data from these males were not used in the analysis. Female fertility was determined based on the number of hatched larvae in each experimental unit. To assess female fertility, eggs from females exposed to pesticide residues were collected, placed in Petri dishes (3.5 cm diameter, 0.7 cm high), sealed with polyvinyl chloride (PVC) film, and kept in a climate-controlled room. The number of hatched larvae in each Petri dish was recorded 4 days after the eggs were transferred to the experimental units.

Data analysis

Generalized linear models (Nelder and Wedderburn 1972) with quasi-binomial, quasi-Poisson and Gaussian distributions were used to analyze the proportion data (female mortality and fertility), counts (female fecundity) and duration (eggs, larvae, protonymphs, deutonymphs, pre-oviposition and oviposition periods, and female and male longevities), respectively. The quality of the fit was determined through a half-normal graph with a simulation envelope (Hinde and Demétrio 1998). In case of significant differences among treatments, multiple comparisons with the Tukey test with the ‘*glht*’ function of the ‘*multcomp*’ package, with adjusted *p* values were conducted. All analyses were performed using the statistical software R v.3.1.3 (R Development Core Team 2015).

The life tables were constructed based on the data for all individuals (including females, males, and individuals that died as immatures) as proposed by Chi (1988). The original data for all individuals were analyzed according to the theoretical model proposed by Chi and Liu (1985), using the TWSEXMSChart program (<http://140.120.197.173/ecology/Download/TWSEX-MSChart.rar>) (Chi 2014). For each treatment, the following parameters were estimated:

$$\begin{aligned} \text{Net reproductive rate } (R_o): R_o &= \sum_{x=0}^{\infty} l_x m_x, \\ \text{Intrinsic rate of increase } (r): \sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x &= 1, \\ \text{Mean generation time } (T): T &= \ln R_o / r, \\ \text{and finite rate of increase } (\lambda): \lambda &= e^r. \end{aligned}$$

The means and standard errors of each parameter were estimated by the bootstrap method, following the procedure described by Huang and Chi (2012). During the bootstrap procedure, the data for each population parameter were resampled 40,000 times. The means for each treatment were compared by paired bootstrap test, based on the difference in confidence interval (Efron and Tibshirani 1993).

Results

Formetanate hydrochloride, dimethoate and phosmet caused 100% female mortality, and therefore were considered highly harmful (Table 2). Females exposed to carbosulfan, diflubenzuron, fenpropathrin, gamma-cyhalothrin, imidacloprid, lambda-cyhalothrin, lambda-cyhalothrin + thiamethoxam, mineral and vegetable oils, spinosad and thiamethoxam suffered mortality rates of 54–78%. In addition to the mortality, mineral and vegetable oils reduced the fecundity of surviving females (Table 2). Regarding the reduction coefficient, all these compounds were moderately harmful to *E. concordis* (Table 2). Beta-cypermethrin, deltamethrin, etofenprox, pyriproxyfen and tebufenozide did not cause significant female mortality, but beta-cypermethrin, deltamethrin, pyriproxyfen and tebufenozide slightly reduced female fecundity, and therefore were considered slightly harmful to *E. concordis* (Table 2).

In addition to the mortality, carbosulfan, formetanate hydrochloride and phosmet were considered highly persistent, because they caused high female mortality for more than 31 days after spraying (Table 3). However, dimethoate was considered moderately

Table 2 Mortality, fecundity, and fertility of *Euseius concordis* females, and reduction coefficient of pesticides used in citrus integrated-production systems

Treatment	Cumulative mortality ^a (%)	Fecundity ^b (no. eggs female ⁻¹)	Fertility ^a (% hatched larvae)	Fecundity effect (R ₁)	Fertility effect (R ₂)	E (%) ^c	IOBC/WPRS class ^d
Control	14 ± 2.45 c	8.1 ± 0.09 a	98.6 ± 0.90 ab	–	–	–	–
Beta-cypermethrin	32 ± 3.74 bc	5.3 ± 0.04 b	97.3 ± 1.39 ab	0.65	0.99	47.1	2
Carbosulfan	78 ± 9.69 ab	6.2 ± 0.08 a	98.7 ± 1.19 ab	0.77	1.00	72.4	3
Formetanate hydrochloride	100 a	–	–	–	–	100.0	4
Deltamethrin	30 ± 5.48 bc	5.5 ± 0.04 ab	95.9 ± 1.20 ab	0.68	0.97	44.5	2
Diflubenzuron	58 ± 3.74 ab	5.7 ± 0.04 ab	92.8 ± 0.76 b	0.70	0.94	63.9	3
Dimethoate	100 a	–	–	–	–	100.0	4
Etofenprox	22 ± 4.90 bc	6.0 ± 0.05 a	98.2 ± 0.55 ab	0.74	1.00	32.1	2
Fenpropathrin	54 ± 2.45 ab	5.5 ± 0.04 ab	87.5 ± 1.65 b	0.68	0.89	63.8	3
Gamma-cyhalothrin	50 ± 6.32 b	5.8 ± 0.02 a	94.8 ± 1.19 b	0.72	0.96	55.9	3
Imidacloprid	62 ± 8.00 ab	6.0 ± 0.11 a	92.0 ± 3.09 b	0.74	0.93	73.8	3
Lambda-cyhalothrin	76 ± 5.10 ab	9.0 ± 0.18 a	91.0 ± 1.88 b	1.11	0.92	61.0	3
Lambda-cyhalothrin + thiamethoxam	72 ± 5.83 ab	7.3 ± 0.14 a	96.0 ± 1.73 ab	0.90	0.97	63.1	3
Mineral oil	58 ± 9.52 ab	5.0 ± 0.09 b	99.5 ± 0.44 a	0.62	1.01	65.1	3
Phosmet	100 a	–	–	–	–	100.0	4
Pyriproxyfen	20 ± 0.63 c	5.6 ± 0.03 ab	96.1 ± 0.91 ab	0.69	0.97	36.7	2
Spinosad	72 ± 3.74 ab	6.4 ± 0.08 a	97.0 ± 0.94 ab	0.79	0.98	67.4	3
Tebuconazole	20 ± 5.48 c	5.7 ± 0.05 ab	98.6 ± 0.76 ab	0.63	1.00	40.8	2
Thiamethoxam	62 ± 5.83 ab	6.0 ± 0.11a	97.8 ± 1.42 ab	0.73	0.99	72.5	3
Vegetable oil	54 ± 9.68 ab	5.2 ± 0.05 b	97.5 ± 1.49 ab	0.72	0.99	67.2	3

Table 2 continued

Treatment	Cumulative mortality ^a (%)	Fecundity ^b (no. eggs female ⁻¹)	Fertility ^a (% hatched larvae)	Fecundity effect (R_1)	Fertility effect (R_2)	E (%) ^c	IOBC/WPRS class ^d
F	23.66	2.49	1.94				
<i>d.f.</i>	19,80	16,68	16,68				
<i>p</i>	<0.001	<0.001	0.03				

^a Means within a column followed by the same letter do not differ significantly (post hoc Tukey test following GLM with quasi-binomial distribution: $p > 0.05$)

^b Means within a column followed by the same letter do not differ significantly (post hoc Tukey test following GLM with quasi-Poisson distribution: $p > 0.05$)

^c Reduction coefficient of insecticides calculated by formula E (%) = $100 - (100 - M_c) \times E_r$, as proposed by Bakker et al. (1992)

^d Toxicity classes according to IOBC/WPRS: 1: harmless ($E < 25\%$ reduction), 2: slightly harmful ($25 \leq E \leq 50\%$ reduction), 3: moderately harmful ($51 \leq E \leq 75\%$ reduction), and 4: harmful ($E > 75\%$ reduction) as proposed by Hassan (1992)

Table 3 Duration of the harmful effects of pesticides used in citrus integrated-production systems in *Euseius concordis* females

Treatment	Mortality (%)/days after spraying (DAS)						IOBC/WPRS class ^b
	3	7	10	17	24	31	
Control	2 ± 1.79 c	0 d	0 d	2 ± 1.79 c	0 c	2 ± 1.79 c	–
Carbosulfan	88 ± 3.35 a	66 ± 4.56 a	66 ± 4.56 b	68 ± 3.35 a	96 ± 2.19 a	74 ± 2.19 a	4
Formetanate hydrochloride	100 a	100 a	100 a	100 a	100 a	100 a	4
Diflubenzuron	8 ± 5.21 c	10 ± 2.83 cd	12 ± 1.79 c	0 c	0 c	2 ± 1.79 c	1
Dimethoate	98 ± 4.43 a	80 ± 2.82 a	80 ± 4.90 a	30 ± 2.83 b	23 ± 6.93 b	22 ± 1.79 b	3
Fenpropathrin	12 ± 3.35 c	4 ± 2.19 cd	6 ± 2.19 d	10 ± 2.83 bc	0 c	8 ± 1.79 bc	1
Gamma-cyhalothrin	38 ± 1.79 b	26 ± 1.79 cd	4 ± 2.19 d	4 ± 2.19 c	0 c	2 ± 1.79 c	2
Imidacloprid	12 ± 1.79 c	14 ± 2.19 c	10 ± 2.83 cd	10 ± 2.83 bc	6 ± 2.19 c	4 ± 2.19 c	1
Lambda-cyhalothrin	30 ± 4.00 b	26 ± 4.56 bc	22 ± 2.19 c	12 ± 3.35 bc	4 ± 2.19 c	0 c	2
Lambda-cyhalothrin + thiamethoxam	34 ± 3.58 b	14 ± 2.19 c	14 ± 2.19 cd	2 ± 1.79 c	0 c	2 ± 1.79 c	2
Mineral oil	8 ± 3.35 c	4 ± 2.19 cd	4 ± 2.19 d	4 ± 2.19 c	2 ± 1.79 c	0 c	1
Phosmet	100 a	100 a	100 a	96 ± 3.58 a	98 ± 1.79 a	94 ± 3.58 a	4
Spinosad	42 ± 7.48 b	46 ± 4.56 b	24 ± 4.56 c	14 ± 2.19 bc	4 ± 2.19 c	0 c	2
Thiamethoxam	8 ± 3.35 c	6 ± 3.67 cd	6 ± 3.58 d	10 ± 2.83 bc	0 c	2 ± 1.79 c	1
Vegetable oil	6 ± 2.19 c	7 ± 3.59 cd	6 ± 2.19 d	4 ± 2.19 c	0 c	2 ± 1.79 c	1
F	38.8	38.7	54.3	41.9	109.0	51.9	
d.f.	14,60	14,60	14,60	14,60	14,60	14,60	
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

^a Means within a column followed by the same letter do not differ significantly (post hoc Tukey test following GLM with quasi-binomial distribution: $p > 0.05$)

^b Persistence class proposed by the IOBC/WPRS: 1: short-life (<5 DAS), 2: slightly persistent (5–15 DAS), 3: moderately persistent (16–30 DAS), and 4: persistent (>30 DAS) (Hassan 1992)

persistent, whereas gamma-cyhalothrin, lambda-cyhalothrin, lambda-cyhalothrin + thiamethoxam and spinosad were slightly persistent, and diflubenzuron, fenpropathrin, imidacloprid, mineral and vegetable oils and thiamethoxam were short-lived in their effects on the predator mite (Table 3).

With respect to their effects on the progeny, none of the pesticides affected the duration of the egg, larva, protonymph and deutonymph stages of *E. concordis* (Table 4). However, in the adults, all compounds (except lambda-cyhalothrin and thiamethoxam) increased the female pre-oviposition period, although they did not affect the oviposition period (Table 5). The number of eggs laid by females (fecundity) was significantly lower in the treatments with carbosulfan, deltamethrin, diflubenzuron, etofenprox, fenpropathrin, gamma-cyhalothrin, mineral and vegetable oils, pyriproxyfen and tebufenozide than in the other treatments (Table 5). On the other hand, female progeny from females treated with thiamethoxam had higher fecundity than in the other treatments (Table 5). The lowest female longevity was observed in the treatments with mineral and vegetable oils (Table 5). Imidacloprid and thiamethoxam induced an increase in female longevity, whereas the other pesticides did not affect female longevity, which was similar to the control (Table 5). Male progeny from females exposed to residues of lambda-cyhalothrin + thiamethoxam, beta-cypermethrin and imidacloprid had higher longevity than those from females treated with carbosulfan, spinosad, etofenprox, and mineral and vegetable oils (Table 5). In spite of

Table 4 Duration of egg, larva, protonymph and deutonymph stages of *Euseius concordis* progeny from females exposed to residual contact with pesticides used in citrus integrated-production systems

Treatment	Duration of mite development stages (days)			
	Egg	Larva	Protonymph	Deutonymph
Control	1.25 ± 0.06 a	1.00 ± 0.00 a	1.13 ± 0.05 a	1.21 ± 0.06 a
Beta-cypermethrin	1.11 ± 0.08 a	1.00 ± 0.00 a	1.18 ± 0.06 a	1.24 ± 0.06 a
Carbosulfan	1.27 ± 0.08 a	1.00 ± 0.00 a	1.26 ± 0.07 a	1.22 ± 0.07 a
Deltamethrin	1.33 ± 0.10 a	1.00 ± 0.00 a	1.28 ± 0.08 a	1.19 ± 0.07 a
Diflubenzuron	1.57 ± 0.09 a	1.00 ± 0.00 a	1.32 ± 0.07 a	1.52 ± 0.11 a
Etofenprox	1.05 ± 0.03 a	1.04 ± 0.03 a	1.35 ± 0.12 a	1.21 ± 0.11 a
Fenpropathrin	1.63 ± 0.09 a	1.00 ± 0.00 a	1.40 ± 0.13 a	1.40 ± 0.13 a
Gamma-cyhalothrin	1.33 ± 0.10 a	1.00 ± 0.00 a	1.48 ± 0.11 a	1.26 ± 0.10 a
Imidacloprid	1.19 ± 0.10 a	1.00 ± 0.00 a	1.33 ± 0.09 a	1.26 ± 0.09 a
Lambda-cyhalothrin	1.41 ± 0.07 a	1.00 ± 0.00 a	1.30 ± 0.10 a	1.56 ± 0.12 a
Lambda-cyhalothrin + thiamethoxam	1.40 ± 0.07 a	1.00 ± 0.00 a	1.23 ± 0.09 a	1.19 ± 0.09 a
Mineral oil	1.32 ± 0.09 a	1.07 ± 0.05 a	1.05 ± 0.03 a	1.02 ± 0.02 a
Pyriproxyfen	1.48 ± 0.10 a	1.00 ± 0.00 a	1.17 ± 0.07 a	1.40 ± 0.10 a
Spinosad	1.26 ± 0.07 a	1.00 ± 0.00 a	1.33 ± 0.10 a	1.33 ± 0.11 a
Tebufenozide	1.27 ± 0.06 a	1.00 ± 0.00 a	1.14 ± 0.07 a	1.22 ± 0.08 a
Thiamethoxam	1.65 ± 0.08 a	1.00 ± 0.00 a	1.32 ± 0.09 a	1.31 ± 0.09 a
Vegetable oil	1.11 ± 0.05 a	1.00 ± 0.00 a	1.07 ± 0.04 a	1.35 ± 0.07 a
F	0.846	0.630	0.407	0.141
<i>d.f.</i>	16,238	16,238	16,238	16,238
<i>p</i>	0.63	0.99	0.98	0.99

Means within a column followed by the same letter do not differ significantly (post hoc Tukey test following GLM with Gaussian distribution: $p > 0.05$)

Table 5 Duration of pre-oviposition and oviposition periods, fecundity and female and male longevities of *Euseius concordis* from females exposed to residual contact with pesticides used in citrus integrated-production systems

Treatment	Pre-oviposition period (days) ^a		Oviposition period (days) ^a		Fecundity ^b (no. eggs/female)		Longevity (days) ^a	
	Female	Male	Female	Male	Female	Male	Female	Male
Control	2.03 ± 0.25 c		8.82 ± 1.66 ab		8.70 ± 2.84 b		12.61 ± 1.84 b	10.72 ± 0.60 ab
Beta-cypermethrin	3.25 ± 0.58 ab		7.62 ± 1.55 ab		6.25 ± 1.19 b		11.75 ± 1.84 b	15.00 ± 1.98 a
Carbosulfan	2.75 ± 0.19 ab		2.42 ± 0.79 b		2.84 ± 0.97 c		6.08 ± 1.07 bc	7.28 ± 0.73 b
Deltamethrin	3.12 ± 0.51 ab		7.62 ± 1.60 ab		5.50 ± 1.32 c		12.37 ± 1.82 b	13.43 ± 1.73 ab
Diflubenzuron	4.10 ± 0.36 a		3.20 ± 0.67 b		2.50 ± 0.47 c		7.40 ± 0.50 bc	13.00 ± 1.15 ab
Etofenprox	3.44 ± 0.29 a		3.28 ± 0.90 b		3.24 ± 0.72 c		8.20 ± 0.94 bc	7.43 ± 0.90 b
Fenpropathrin	3.71 ± 0.55 a		8.86 ± 1.89 ab		5.86 ± 1.39 c		14.07 ± 2.14 ab	11.17 ± 1.26 ab
Gamma-cyhalothrin	2.67 ± 0.21 ab		6.00 ± 1.86 b		5.33 ± 1.79 c		9.22 ± 1.86 bc	11.92 ± 0.83 ab
Imidacloprid	3.10 ± 0.36 ab		14.80 ± 1.86 a		13.30 ± 2.16 ab		20.50 ± 1.94 a	15.25 ± 1.84 a
Lambda-cyhalothrin	2.12 ± 0.21 bc		6.71 ± 1.53 b		10.24 ± 1.39 b		10.96 ± 0.96 b	8.56 ± 1.97 ab
Lambda-cyhalothrin + thiamethoxam	2.46 ± 0.14 b		12.27 ± 2.33 a		12.08 ± 1.93 ab		12.96 ± 2.00 ab	16.00 ± 2.94 a
Mineral oil	2.63 ± 0.14 ab		2.10 ± 1.16 b		2.09 ± 1.33 c		5.36 ± 1.43 c	5.84 ± 1.35 b
Pyriproxyfen	3.46 ± 0.44 a		4.54 ± 1.16 b		3.61 ± 1.18 c		8.92 ± 1.49 bc	14.87 ± 1.72 ab
Spinosad	2.56 ± 0.15 ab		6.31 ± 1.32 b		6.31 ± 1.79 b		9.81 ± 1.71 bc	6.00 ± 0.79 b
Tebufozozide	3.53 ± 0.29 a		4.29 ± 0.93 b		4.75 ± 0.66 c		10.82 ± 1.09 bc	8.47 ± 1.68 ab
Thiamethoxam	2.18 ± 0.21 bc		13.25 ± 2.09 a		16.44 ± 2.21 a		15.50 ± 1.75 a	12.87 ± 1.52 ab
Vegetable oil	2.67 ± 0.24 ab		2.00 ± 0.82 b		2.36 ± 0.88 c		5.50 ± 1.04 c	5.80 ± 0.66 b
F	3.49		6.44		6.85		11.29	5.02
d.f.	16,238		16,238		16,238		16,238	16,238
p	<0.001		<0.001		<0.001		<0.001	<0.001

^a Means within a column followed by the same letter do not differ significantly (post hoc Tukey test following GLM with Gaussian distribution; $p > 0.05$)

^b Means within a column followed by the same letter do not differ significantly (post hoc Tukey test following GLM with quasi-Poisson distribution; $p > 0.05$)

Table 6 Estimated life-table parameters of *Euseius concordis* progeny from females exposed to pesticide residues used in citrus integrated-production systems

Treatment	Net reproductive rate (R_o) (female female ⁻¹)	Mean generation time (T) (days)	Intrinsic rate of increase (r) (female female ⁻¹ day ⁻¹)	Finite rate of increase (λ) (female female ⁻¹ day ⁻¹)
Control	3.6 ± 0.87 b	16.1 ± 0.56 a	0.08 ± 0.002 b	1.08 ± 0.002 b
Beta-cypermethrin	3.2 ± 0.92 b	14.7 ± 1.14 a	0.07 ± 0.003 b	1.07 ± 0.003 b
Carbosulfan	1.7 ± 0.44 bc	13.3 ± 1.20 b	0.04 ± 0.003 c	1.04 ± 0.004 c
Deltamethrin	1.8 ± 0.69 bc	14.7 ± 0.44 b	0.04 ± 0.003 c	1.04 ± 0.003 c
Diflubenzuron	1.4 ± 0.27 c	11.2 ± 1.94 b	0.03 ± 0.003 c	1.04 ± 0.004 c
Etofenprox	1.3 ± 0.42 c	13.1 ± 1.77 b	0.02 ± 0.002 c	1.02 ± 0.003 c
Fenpropathrin	2.7 ± 0.80 bc	16.6 ± 0.83 a	0.06 ± 0.002 b	1.06 ± 0.002 b
Gamma-cyhalothrin	1.5 ± 0.67 bc	13.5 ± 1.77 b	0.03 ± 0.002 c	1.03 ± 0.004 c
Imidacloprid	8.3 ± 2.09 a	16.3 ± 0.73 a	0.13 ± 0.002 a	1.20 ± 0.002 a
Lambda-cyhalothrin	4.2 ± 0.85 ab	13.0 ± 0.45 b	0.11 ± 0.002 a	1.10 ± 0.002 a
Lambda-cyhalothrin + thiamethoxam	6.6 ± 1.28 a	13.5 ± 0.49 b	0.14 ± 0.001 a	1.20 ± 0.002 a
Mineral oil	1.4 ± 0.44 c	9.6 ± 1.46 c	0.03 ± 0.003 c	1.03 ± 0.004 c
Pyriproxyfen	1.7 ± 0.53 bc	13.3 ± 2.25 b	0.04 ± 0.002 c	1.04 ± 0.003 c
Spinosad	2.9 ± 0.86 bc	13.2 ± 1.12 b	0.08 ± 0.002 b	1.07 ± 0.003 b
Tebufenozide	2.4 ± 0.52 bc	12.5 ± 0.56 b	0.07 ± 0.002 b	1.07 ± 0.002 b
Thiamethoxam	7.3 ± 1.63 a	13.2 ± 0.53 b	0.15 ± 0.002 a	1.20 ± 0.002 a
Vegetable oil	1.3 ± 0.39 c	9.6 ± 1.46 c	0.02 ± 0.002 c	1.02 ± 0.003 c

Means within a column followed by the same letter do not differ significantly (paired bootstrap test: $p > 0.05$)

these variations among the pesticides, no significant difference was observed in male longevity when these compounds were compared to the control.

Our results showed that imidacloprid, lambda-cyhalothrin, lambda-cyhalothrin + thiamethoxam, and thiamethoxam led to a higher net reproductive rate (R_o), intrinsic rate of increase (r) and finite rate of increase (λ) of the mite compared to the other treatments (Table 5). However, diflubenzuron, etofenprox, and mineral and vegetable oils substantially reduced the R_o , r and λ of *E. concordis* from the values in the control treatment (Table 6). All the other pesticides tested did not affect R_o , r and λ of the mite, which were similar to the control. However, all pesticides except beta-cypermethrin, fenpropathrin and imidacloprid reduced the mean generation time (T) of *E. concordis*. The greatest reductions in T were observed in mites from females exposed to residues of mineral and vegetable oils (Table 6).

Discussion

Formetanate hydrochloride, dimethoate and phosmet proved to be highly harmful to *E. concordis*. In assays with other species, formetanate hydrochloride proved to be highly toxic to females of *Neoseiulus californicus* (McGregor) (Phytoseiidae) and *Phytoseiulus macropilis* (Banks) (Phytoseiidae) (Poletti et al. 2008), whereas dimethoate induced high

mortality in females of *Amblyseius cucumeris* (Oudemans) (Phytoseiidae), *Hypoaspis aculeifer* (Canestrini) (Mesostigmata: Laelapidae), *Hypoaspis miles* (Berlese) (Mesostigmata: Hypoaspidae) and *Agistemus brasiliensis* (Matioli, Ueckermann and Oliveira) (Prostigmata: Stigmaeidae) exposed to residual contact with these pesticides (Miles and Dutton 2003; Silva et al. 2009). Phosmet was considered highly harmful to females of *Iphiseiodes zuluagai* (Denmark & Muma) (Phytoseiidae) (Reis et al. 1998) and *Anystis baccarum* (L.) (Mesostigmata: Anystidae) (Laurin and Bostanian 2007), demonstrating that these pesticides are not selective for the predatory mites. Besides the acute toxicity, formetanate hydrochloride and phosmet were considered highly persistent (mortality > 94%, 31 DAS), whereas dimethoate was considered moderately persistent (mortality 23%, 24 DAS). Therefore, these compounds are incompatible with *E. concordis*, because they can reduce the population levels and effectiveness of this predator mite as a biocontrol agent in citrus orchards. Furthermore, the prolonged residual period of these pesticides can also negatively affect the maintenance and recolonization of this predator in agroecosystems.

Carbosulfan, diflubenzuron, fenpropathrin, gamma-cyhalothrin, imidacloprid, lambda-cyhalothrin, lambda-cyhalothrin + thiamethoxam, mineral and vegetable oils, spinosad and thiamethoxam caused mortality of 42–78% of females exposed to residues of these compounds. Similar results were observed for *Galendromus occidentalis* (Nesbitt) (Phytoseiidae) and *I. zuluagai* treated with lambda-cyhalothrin (Beers and Schmidt 2014; Zanardi et al. 2017), and for *P. macropilis* exposed to fenpropathrin residues (Silva and Oliveira 2006). Carbosulfan and mineral and vegetable oils were highly harmful (100% mortality) to *I. zuluagai* females (Reis et al. 1998), whereas imidacloprid was moderately harmful to females of *Tydeus californicus* (Banks) (Prostigmata: Tydeidae) and *Neoseiulus fallacis* (Garman) (Phytoseiidae) (Castagnoli et al. 2005; Villanueva and Walgenbach 2005) exposed to residual contact with these pesticides. Likewise, thiamethoxam was moderately toxic to females of *Phytoseiulus persimilis* Athias-Henriot (Phytoseiidae) and *Kampimodromus aberrans* (Oudemans) (Phytoseiidae) (Duso et al. 2008; Tirello et al. 2013; Duso et al. 2014). In addition to the moderate acute toxicity, these pesticides caused different sublethal effects on *E. concordis*. Females exposed to residues of carbosulfan, diflubenzuron, fenpropathrin, gamma-cyhalothrin, mineral and vegetable oils, and thiamethoxam showed significant reduction in the number of eggs laid. In addition, all these compounds (except for lambda-cyhalothrin and thiamethoxam) prolonged the pre-oviposition period of female offspring of *E. concordis*. Mineral and vegetable oils also reduced the longevity of female offspring of this predator mite. Reductions in reproductive parameters (especially fecundity) have been previously observed in predatory mites exposed to pyrethroids (Poletti et al. 2008; Beers and Schmidt 2014; Zanardi et al. 2017), neonicotinoids (Bostanian et al. 2009, 2010; Pozzebon et al. 2015; Zanardi et al. 2017), spinosad (Duso et al. 2014), and residues of mineral and vegetable oils (Silva et al. 2015). Surprisingly, in this study we observed that female offspring from females treated with imidacloprid, lambda-cyhalothrin and thiamethoxam had a significant increase in fecundity. Increases in the reproductive rate of mites after pesticide exposure have often been recorded in various mite species (Castagnoli et al. 2005; Barati and Hejazi 2015). In the field of toxicology, this effect is known as hormesis, a phenomenon characterized by a biphasic dose response, with a low-dose stimulation or beneficial effect and a high-dose inhibitory effect (Mattson 2009). Therefore, in this study, we have demonstrated the stimulant effect caused by imidacloprid, lambda-cyhalothrin and thiamethoxam on female offspring of *E. concordis*. This stimulant effect may be associated with changes in the physiology of the mites that increase the production of vitellin, and a resulting increase in

the number of eggs (Zeng and Wang 2010). Therefore, additional studies should be carried out to assess the effects of these compounds on the physiological processes involved in the reproduction of predatory mites.

Although these insecticides were considered moderately harmful to *E. concordis* in our acute toxicity assay, gamma-cyhalothrin, lambda-cyhalothrin, lambda-cyhalothrin and spinosad were slightly persistent, whereas diflubenzuron, fenpropathrin, imidacloprid, mineral and vegetable oils and thiamethoxam were considered short-lived. Durations of harmful activity similar to those obtained in the present study were observed in females of *Euseius stipulatus* (Athias-Henriot) (Phytoseiidae) exposed to imidacloprid (Jacas and Garcia-Marí 2001), spinosad (San-Andrés et al. 2006), and mineral oil (Pascual-Ruiz and Urbaneja 2006). The short duration of the harmful effects (low persistence) of these pesticides reduces their impact on individuals of *E. concordis* present in the production areas, which enables recolonization and the establishment of an ecological equilibrium in agroecosystems. The low persistence may also facilitate mass releases of the predator a short time after application of the pesticides. This possibility was explored by Argolo et al. (2013), who evaluated the effect of imidacloprid on *P. persimilis* and found that this pesticide did not affect the predator's efficacy in controlling pest mites in clementine nurseries. Moreover, the combination of *P. persimilis* releases with spinosad applications has yielded positive results, due to the low residual persistence of this pesticide (Holt et al. 2006). Therefore, field studies should be carried out to assess the impacts of these compounds on predatory mites in integrated pest-management programs.

In contrast, beta-cypermethrin, deltamethrin, etofenprox, pyriproxyfen and tebufenozide were considered slightly harmful to *E. concordis*. Moreover, beta-cypermethrin, pyriproxyfen and tebufenozide increased the pre-oviposition period and reduced the fecundity of the offspring females. Deltamethrin was also considered harmless to *N. californicus* females (Silva and Oliveira 2006), whereas beta-cypermethrin was slightly harmful larvae and harmless to females of *N. californicus* (Poletti et al. 2008). Kaplan et al. (2012) found that pyriproxyfen was harmless to the larvae and slightly harmful to the females of *N. californicus*. Likewise, tebufenozide was slightly harmful to *Amblyseius andersoni* (Chant) and *Typhlodromus pyri* Scheuten (both Phytoseiidae) (Pozzebon et al. 2015). Among the compounds tested here, beta-cypermethrin, deltamethrin, etofenprox, pyriproxyfen and tebufenozide caused the lowest lethal and sublethal effects on *E. concordis*, and therefore they are considered more suitable to use in pest management programs that aim at the conservation of this biocontrol agent in citrus production systems.

Regarding the population parameters estimated for *E. concordis* offspring, our results indicated that diflubenzuron, etofenprox, and mineral and vegetable oils reduced the net reproductive rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), and mean generation time (T) of *E. concordis*. On the other hand, imidacloprid, lambda-cyhalothrin and thiamethoxam induced significant increases in R_0 , r , and λ of *E. concordis* offspring, whereas the effects of beta-cypermethrin, carbosulfan, deltamethrin, fenpropathrin, gamma-cyhalothrin, lambda-cyhalothrin, pyriproxyfen, spinosad and tebufenozide were similar to the control treatment. Although diflubenzuron, etofenprox, and mineral and vegetable oils reduced the population parameters, the mean values of r and λ were positive, indicating that the *E. concordis* population was still able to increase. According to Van Lenteren (2000), it is desirable that the biological control agent r be equal to or greater than the target-pest population r . Higher r values are important because they indicate a higher reproductive potential of the biological control agent (Moscardini et al. 2013). It should be emphasized, however, that this study was performed under laboratory conditions, where the mites were exposed to pesticide residues for a long period, producing extreme results

that may not occur under field conditions. In the field, *E. concordis* can locate areas that are free of residues, decreasing the risk of contamination with these compounds. Furthermore, the biological action of these pesticides could be significantly reduced due to the biotic and abiotic factors that act to degrade the active ingredient (Daam and Van den Brink 2010; Hulbert et al. 2011). Therefore, semi-field and field studies are needed to assess the pesticide effects on *E. concordis* density and population dynamics and to determine their compatibility with this predatory mite in IPM programs.

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