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PEST MANAGEMENT

Lethal and Sublethal Toxicity of Insecticides to the Lacewing *Ceraeochrysa Cubana*

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Abstract

The lethal and sublethal effects of 11 insecticides on the predator *Ceraeochrysa cubana* (Hagen) were assessed under laboratory conditions. First-instar larvae and adults ≤ 48 h old were sprayed with the highest insecticides doses allowed to control *Diaphorina citri* Kuwayama in the citrus crop. The survival and duration rates of the different development stages, sex ratio, pre-oviposition period, fecundity, and fertility of the insects were evaluated. In the larval bioassay, chlorpyrifos and malathion had lethal effect which none larvae survived. Azadirachtin, lambda-cyhalothrin + chlorantraniliprole, lambda-cyhalothrin + thiamethoxam, and thiamethoxam had lethal and sublethal effects that did not allow to estimate the life table parameters because the low number of couples formed. Esfenvalerate, imidacloprid WG and SC, phosmet, and pyriproxyfen had sublethal effects which were reflected in the net reproductive rate and in the intrinsic rate of natural increase. In bioassay using adults, none of the individuals survived in the chlorpyrifos, lambda-cyhalothrin + chlorantraniliprole, lambda-cyhalothrin + thiamethoxam, malathion, or thiamethoxam treatments, and the azadirachtin, esfenvalerate, imidacloprid WG and SC, phosmet, and pyriproxyfen treatments were significantly lower compared to the control. None of the insecticides was harmless to first-instar larvae and adults of *C. cubana* under laboratory conditions showing their potential to reduce the efficiency of this predator.

Introduction

Insecticides are one of the main tools used in citrus pest management to reduce the pest population density below the level of economic damage (Belasque *et al* 2010, Grafton-Cardwell *et al* 2013). The variety of chemical groups and active principles of licensed insecticides used in Brazilian citriculture is large (MAPA 2016). These insecticide spraying amounts have increased in last years due to Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Belasque *et al* 2010). This psyllid is a key pest in citrus because it is a vector of bacteria "*Candidatus* Liberibacter americanus" and "*Candidatus* Liberibacter asiaticus" associated with huanglongbing (HLB), a major disease in world citriculture (Teixeira *et al* 2005, Grafton-Cardwell *et al* 2013).

In addition to chemical control, the biological control is another important tactic in integrated pest management (IPM) to maintain pest populations below levels that cause economic damage (Grafton-Cardwell *et al* 2013). In citrus, lacewings are one of the major natural enemies (Cortez-Mondaca *et al* 2016). They are generalists, feeding on a wide range of phytophagous insects, and occur naturally in this crop throughout the year (Tauber *et al* 2000b, Godoy *et al* 2010, Pappas *et al* 2011). Among the lacewing species found in citrus orchards, *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae) stands out for its predatory capability, high reproductive rate, wide geographical distribution throughout the Americas, and association with several crops (Lopez-Arroyo *et al* 1999, Tauber *et al* 2000a, Freitas & Penny 2001).

The greatest difficulty in IPM programs is to integrate chemical with biological control methods, combining the advantages of both (Biondi *et al* 2015, Garzón *et al* 2015). Pesticides control the target pest but on the other hand, they may reduce the populations of natural enemies, leading to undesirable effects, including the resurgence of the target pest, outbreak of secondary pests, and selection of populations that are resistant to insecticides (Devine & Furlong 2007). In addition to acute toxicity (lethal effect), insecticides can also cause sublethal effects on natural enemies, triggering physiological and behavioral changes that affect the development, sex ratio, fertility, fecundity, mating, longevity, mobility, orientation, and foraging capacity, which may reduce and/or imped the activity of these biological control agents in agroecosystems (Desneux *et al* 2007, He *et al* 2012).

As it is known, the use of pesticides affects the beneficial insects present on the agroecosystem, including lacewings (Rugno *et al* 2015, Ono *et al* 2017). Understanding pesticide impact on performance of the natural enemies, lethal and sublethal effects must be evaluated (Desneux *et al* 2006a). Sublethal effects are an important feature to be considered because small changes which do not kill the insect can affect its development and reproduction, reflecting on demographic parameters (Desneux *et al* 2006b). In order to understand the pesticide effects on lacewings present in citrus agroecosystem in a better way, we evaluated the lethal and sublethal effects (duration and survival of immature stages, sex ratio, pre-oviposition period, daily and total fecundity, and fertility of females) of 11 insecticides recommended for *D. citri* by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA 2016) on first-instar larvae and adults of *C. cubana*, which are the most susceptible stage of this predator (Mandour 2009).

Material and Methods

All bioassays were carried out in a climate-controlled room (temperature 25 ± 2°C, relative humidity (RH) 70 ± 10% and photoperiod of 14:10 L:D h), following a completely randomized design.

Insects

The *C. cubana* colony used in the trials was obtained from 50 adults collected in an experimental citrus orchard at the 'Luiz de Queiroz' College of Agriculture, University of São Paulo (ESALQ/USP), located in Piracicaba, São Paulo, Brazil (22°43′01″S, 47°37′04″W). The adults were identified by Professor Sergio de Freitas from the Universidade Estadual Paulista "Julio de Mesquita Filho" (FCAV/UNESP). They were transferred to PVC cages (30 cm height × 15 cm diameter) which were covered with white bond paper for oviposition and closed at the upper end with net secured with an elastic band. The bottom of each cage was supported on a Petri dish (15 cm diameter), covered with filter-paper discs. The adults were fed with a mixture of honey and brewer's yeast (1:1, *v*:*v*). The ecloded larvae were kept in individual glass tubes (2.5 cm diameter × 8.5 cm height) and fed with eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) rendered unviable with an ultraviolet light source (UV) as described by Stein & Parra (1987). The fourth generation of laboratory was used in order to perform the bioassays.

Insecticides

Eleven insecticides licensed by the MAPA (2016) for the management of *D. citri* in Brazil were assessed on first-instar larvae and adults of *C. cubana*. The insecticides and field concentrations (g a.i. L^{-1}) assessed were as follows: three neonicotinoids, thiamethoxam (Actara® 25 WG—0.025, Syngenta Crop Protection Ltda., São Paulo, SP), and imidacloprid in two formulations (Evidence® 700 WG—0.032 and Provado® 20 SC—0.04, Bayer CropScience, São Paulo, SP); three organophosphates, phosmet (Imidan® 50 WP—0.25, Cross Link Ltda., Barueri, SP), chlorpyrifos (Lorsban® 48 BR—0.72, Dow AgroSciences Ltda., São Paulo, SP), and malathion (Malathion® 100 EC—1.5, Cheminova Ltda., São Paulo, SP); two insecticides each one composed of two active ingredients, lambda-cyhalothrin + chlorantraniliprole (Ampligo®—0.015 + 0.03, Syngenta) and lambda-cyhalothrin + thiamethoxam (EngeoPleno®—0.016 + 0.021, Syngenta); a juvenile hormone analogue, pyriproxyfen (Tiger® 10 EC—0.0625, Sumitomo Ltda., Sao Paulo, SP); a biopesticide, azadirachtin (Azamax® 1.2 EC—0.03, UPL Brazil, Campinas, SP); and a pyrethroid, esfenvalerate (Sumidan® SC—0.125, Sumitomo).

Effects on C. cubana larvae

The direct contact of insecticides on *C. cubana* was evaluated simulating a field pulverization. Forty first-instar larvae ≤ 48 h old for each treatment were used. This instar was chosen because it is more sensible to insecticides (Bueno & Freitas 2001). The larvae were transferred in individual Petri dishes (8.5 cm diameter × 0.5 cm height) and sprayed with 2 mL of a solution of each treatment in a Potter tower. The pressure was adjusted to 68.64 kPa, resulting in deposition of 1.8 ± 0.1 mg cm^{-2} of fresh residues, which is consistent with the criteria established by the International Organization for Biological and Integrated Control/West Palearctic Regional Section (IOBC/WPRS) (Hassan *et al* 1994). Distilled water was used as the control treatment. After spraying, the larvae were placed in individual glass tubes (2.5 cm diameter × 8.5 cm height) sealed with polyvinyl chloride film, and fed ad libitum with *E. kuehniella* eggs as previously described. The insects were kept in the same tubes until adult emergency. From these adults, couples were formed in a number depending on the quantity of males and females obtained per treatment. These couples were placed in cages and fed as described above.

The lethal effect was calculated based on larval mortality. The sublethal effects were evaluated daily until the death of all individuals. For immature, stage duration and survival were evaluated, and for the adults, sex ratio, pre-oviposition period (period from female emergence until its first oviposition), daily and total fecundity (number of eggs per female), and fertility were evaluated. Adults were sexed based on the abdomen segment morphology (Freitas & Penny 2001). The fertility was assessed from 100 eggs collected from each female after the second egg-laying and placed on individual ELISA plates (EasyPath Ltda., São Paulo, SP), coated with PVC film, and stored in a climate-controlled room as it was described above.

Effect on C. cubana adults

To assess the insecticide effects on adults, 12 adult couples ≤ 48 h old were used for each treatment. The adults were initially anesthetized with CO_2 for 10 s and then sprayed with 2 mL of solution in a Potter tower as described in larva bioassay. After spraying, couples were formed and placed in PVC cages.

The lethal effect (mortality) on adults for each treatment was evaluated 24 h after the insecticide spraying. The surviving adults in each treatment were daily watched out until dead to assess the pre-oviposition period, daily and total fecundity, and fertility. Fertility was evaluated for 100 eggs from each female, using the same methodology used in larva bioassay.

Toxicological classes

Based on the lethal and sublethal effects (fecundity and fertility of females), we calculated the total effect for each insecticide, using the formula proposed by Van de Veire *et al* (1996):

$$E\ (\%) = 100-(100-\mathrm{MC}) \times \mathrm{Er}$$

where MC is the first-instar larval mortality corrected by the formula proposed by Abbott (1925); "*n* in *T* after treatment" is the number of insects after sprayed the treatment; and "*n* in Co after treatment" belongs to the number of insects after sprayed the control:

$$\mathrm{MC} = \left(1-\frac{n \text{ in } T \text{ after treatment}}{n \text{ in Co after treatment}}\right) \times 100$$

and Er is the effect on reproduction:

$$\mathrm{Er} = \left(\frac{\text{fecundity in treatment}}{\text{fecundity in control}}\right) \times \left(\frac{\text{fertility in treatment}}{\text{fertility in control}}\right)$$

Based on the total effect, the insecticides were classified according to the toxicological classes suggested by the IOBC/WPRS for laboratory tests (Hassan *et al* 1994): class 1 = harmless ($E \leq 30\%$), class 2 = slightly harmful ($30\% < E \leq 79\%$), class 3 = moderately harmful ($80\% \leq E \leq 99\%$), and class 4 = harmful ($E > 99\%$).

Life table

From the data obtained in the bioassay with *C. cubana* larvae (survival and duration of young stages, sex ratio, female pre-oviposition period, fecundity, and fertility), we estimated the net reproductive rate (R_o), intrinsic rate of natural increase (r_m), finite rate of increase (λ), mean generation time (T), and population doubling time (Td) of the predator using the Jackknife method (Meyer *et al* 1986). Means were compared using a two-sample *t* test ($p < 0.05$), following the protocol for analysis suggested by Maia *et al* (2000), using SAS software (SAS 2003).

Data analysis

Generalized linear models (GLM) (Nelder & Wedderburn 1972) with quasi-binomial distribution were used to analyze data of larval and pupal survival and quasi-Poisson distributions were used to analyze larval and pupal duration, pre-oviposition period, fecundity, and fertility. The quality of the adjustment was determinate through a half-normal graph with a simulation envelope (Hinde & Demétrio 1998). When significant differences were found, multiple comparisons with the Tukey test ($p < 0.05$) were made using the "*glht*" function of the "*multcomp*" package, with adjusted *p* values. All analyses were performed using the statistical software R® (R 2010).

Results

Effects on C. cubana larvae

We observed differences in the survival of *C. cubana* larvae when exposed to the insecticides ($F = 53.59$; df = 11, 36; $p < 0.001$) (Table 1). Azadirachtin, esfenvalerate, imidacloprid SC, phosmet, and pyriproxyfen did not reduce larval survival compared to control (Table 1). However, no larvae survived the chlorpyrifos and malathion treatments 24 h after spraying, and larvae treated with imidacloprid WG, lambda-cyhalothrin + chlorantraniliprole, lambda-cyhalothrin +

Table 1 Mean larval and pupal survival (± SE) and sex ratio of *Ceraeochrysa cubana* when first-instar larvae were sprayed with insecticides.

Treatments	Concentration (g a.i. L^{-1})	Larval survival (%)	Pupal viability (%)	Sex ratio
Azadirachtin	0.03	87.50 ± 4.80a	37.00 ± 12.11b	0.00b
Chlorpyrifos	0.72	0.00 ± 0.00c	–	–
Esfenvalerate	0.125	62.50 ± 4.79a	64.00 ± 12.84ab	0.55a
Imidacloprid SC	0.04	62.50 ± 4.79a	60.00 ± 11.22ab	0.20b
Imidacloprid WG	0.032	50.00 ± 12.25b	78.95 ± 17.80a	0.44a
Lambda-cyhalothrin + chlorantraniliprole	0.015 + 0.03	10.00 ± 2.50c	–	–
Lambda-cyhalothrin + thiamethoxam	0.016 + 0.021	20.00 ± 4.08b	33.50 ± 0.00b	0.00b
Malathion	1.5	0.00 ± 0.00c	–	–
Phosmet	0.25	77.50 ± 4.79a	67.74 ± 7.27ab	0.35a
Pyriproxyfen	0.0625	90.00 ± 4.08a	75.76 ± 10.73a	0.48a
Thiamethoxam	0.025	17.50 ± 6.30b	42.86 ± 5.56ab	0.00b
Control	–	97.50 ± 2.50a	74.36 ± 9.35a	0.58a

Means (± SE) followed by the same letter in a column do not differ significantly (GLM with quasi-binomial distribution, followed by post hoc Tukey test; $p < 0.05$).

thiamethoxam, and thiamethoxam showed significantly low survival rates (Table 1). The development time of larvae that survived was not affected by the insecticides ($F = 0.45$; df = 9, 23; $p = 0.16$) (Fig 1).

The viability of pupae formed from larvae exposed to insecticides differed ($F = 54.97$; df = 9, 26; $p < 0.001$) (Table 1). Azadirachtin and lambda-cyhalothrin + thiamethoxam reduced pupal viability, while lambda-cyhalothrin + thiamethoxam increased the duration of the pupal stage ($F = 13.32$; df = 8, 22; $p < 0.001$) (Fig 1).

It was not possible evaluating the sex ratio in the chlorpyrifos, lambda-cyhalothrin + chlorantraniliprole, or malathion treatments, because no adults emerged. In the azadirachtin, lambda-cyhalothrin + thiamethoxam, and thiamethoxam treatments, only males emerged ($\chi^2 = 2.20$; df = 6; $p < 0.001$) (Table 1).

The pre-oviposition period (5.5 to 6 days) ($F = 0.43$; df = 5, 40; $p = 0.94$), longevity (43.42 to 49.51 days) ($F = 0.16$; df = 5, 40; $p = 0.97$), total oviposition (524.83 to 613.31 eggs) ($F = 0.56$; df = 5, 40; $p = 0.72$), daily fecundity ($F = 0.54$; df = 5, 40; $p = 0.74$), and fertility ($F = 0.28$; df = 5, 40; $p = 0.92$) did not significantly differ in those treatments that allowed the formation of couples (Table 2).

Life table

The treatments that allowed a calculation of life table parameters in the larval bioassays were esfenvalerate, imidacloprid WG and SC, phosmet, and pyriproxyfen. The net reproductive rate (R_o) was reduced in all the treatments when it was compared to the control. All the insecticides evaluated reduced the intrinsic rate of natural increase (r_m) except esfenvalerate (Table 3).

The larvae treated with imidacloprid WG and phosmet showed the longest mean generation times (Table 3). All treatments lengthened the population doubling time (Td) compared to the control (Table 3).

The finite rate of increase (λ) was significantly lower in the imidacloprid WG and SC, phosmet, and pyriproxyfen treatments, while esfenvalerate did not affect this parameter, being similar to the control (Table 3). Considering the life table as a whole, only esfenvalerate did not affect the development of *C. cubana*.

Effects on C. cubana adults

No adults survived the chlorpyrifos, lambda-cyhalothrin + chlorantraniliprole, lambda-cyhalothrin + thiamethoxam, malathion, or thiamethoxam treatments 24 h after spraying (Table 4). Adult survival in the azadirachtin, esfenvalerate, imidacloprid WG and SC, phosmet, and pyriproxyfen treatments was lower compared to the control ($F = 35.15$; df = 11, 28; $p < 0.001$) (Table 4). Due to the low number of surviving adults, it was not possible to perform statistical analyses for pre-oviposition, fertility, daily fecundity, and total fecundity.

Classification of insecticides

Based on the total effect of insecticides applied to larvae, azadirachtin, chlorpyrifos, lambda-cyhalothrin + chlorantraniliprole, lambda-cyhalothrin + thiamethoxam, malathion, and thiamethoxam were harmful (class 4), while esfenvalerate, imidacloprid WG and SC, phosmet, and pyriproxyfen were slightly harmful to the predator (class 2) (Table 2).

When insecticides were sprayed on the *C. cubana* adults, imidacloprid WG, phosmet, and pyriproxifen were

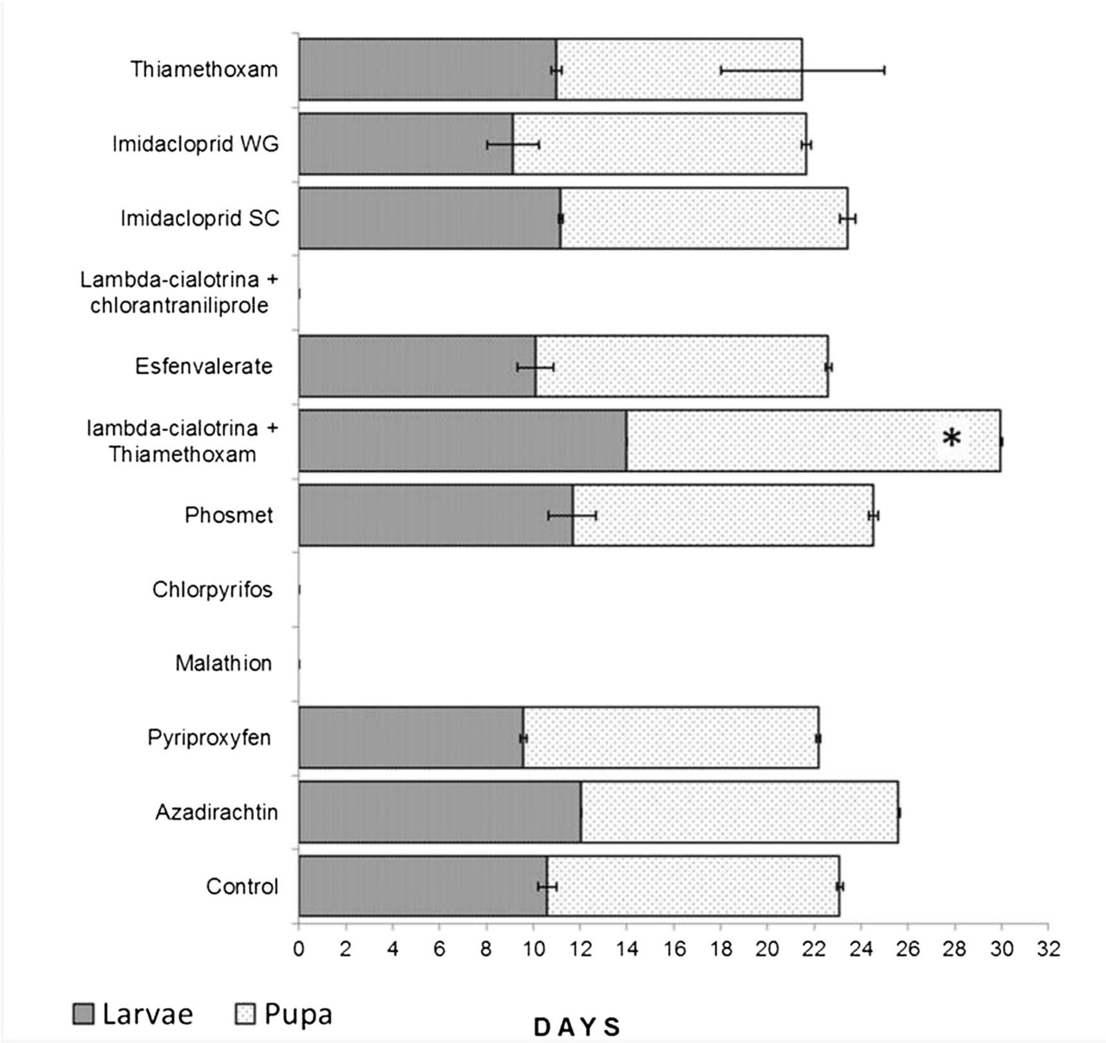

Fig 1 Mean duration of larval and pupal stages (days) of *Ceraeochrysa cubana* when first-instar larvae were sprayed with insecticides. Asterisk indicates statistical difference between treatments by post hoc Tukey test ($p \leq 0.05$).

moderately harmful (class 3) while the other insecticides were classified as harmful for *C. cubana* (Table 4).

Discussion

The 11 tested insecticides recommended for the management of *D. citri* were toxic for first-instar larvae and adults of *C. cubana*. In general, larval stages of lacewings are commercialized for inoculative releases in biological control of a variety of pests. In the case of *C. cubana*, larvae are the only predatory phase and the side effects found in this work may compromise its efficiency as natural enemy. Additionally, the insecticides may also limit the lacewing population growth due to the negative effects on the adults shown in this work, because they are responsible for the reproduction and spread of the species between crops (Daane & Yokota 1997, Tauber *et al* 2000b, Michaud 2001).

Between the three organophosphate insecticides tested, chlorpyrifos and malathion showed high lethal effect on first-instar larvae and adults of *C. cubana* reducing the survival of individuals 24 h after spraying. Chlorpyrifos and malathion are highly toxic to other species of lacewings and to the main parasitoid of *D. citri*, *Tamarixia radiata* (Waterston) (Carvalho *et al* 2003, Costa *et al* 2003, Silva *et al* 2006, Cosme *et al* 2009, Cordeiro *et al* 2010, Castilhos *et al* 2011, Sabry & El-Sayed 2011, Neetan & Aggarwal 2013, Beloti *et al* 2015); therefore, these insecticides must be carefully used in IPM programs. Phosmet was less toxic to *C. cubana*, showing lethal effects on adults and sublethal effects on larvae that could be observed on the life table parameters such as reduction of r_m, limiting the population growth of the predator. These results reinforce the importance of life table analysis in the study of insecticide selectivity. Phosmet already showed some side effects on lacewings in residual-contact bioassay (Giolo *et al* 2009). A possible explanation about why

Table 2 Effects on daily fecundity and fertility, total effect, and IOBC classes when first-instar larvae of *Ceraeochrysa cubana* were sprayed with insecticides.

Treatments	Concentration (g a.i. L^{-1})	Number of couples	Mean daily fecundity[4]	Fertility (%)[5]	MC[1]	*E* (%)[2]	Class (IOBC)[3]
Azadirachtin	0.03	–	–	–	55.17	–	4
Chlorpyrifos	0.72	–	–	–	100	–	4
Esfenvalerate	0.125	6	10.78 ± 2.54a	91.00 ± 2.35a	44.83	56.60	2
Imidacloprid SC	0.04	6	13.55 ± 0.81a	90.00 ± 0.00a	48.28	49.42	2
Imidacloprid WG	0.032	7	14.90 ± 1.69a	89.00 ± 1.23a	55.17	52.32	2
Lambda-cyhalothrin + chlorantraniliprole	0.015 + 0.03	–	–	–	100	–	4
Lambda-cyhalothrin + thiamethoxam	0.016 + 0.021	–	–	–	96.55	–	4
Malathion	1.5	–	–	–	100	–	4
Phosmet	0.25	7	9.87 ± 2.56a	88.86 ± 1.27a	27.50	49.01	2
Pyriproxyfen	0.0625	10	10.76 ± 1.75a	89.45 ± 1.13a	6.90	31.14	2
Thiamethoxam	0.025	–	–	–	89.66	–	4
Control	–	14	13.80 ± 1.71a	90.36 ± 1.05a	–	–	–

[1] Mortality corrected by Abbott's formula (1925). [2] Total effect $E = 100 - (100 - Mc) \times Er$. [3] Toxicological class proposed by IOBC/WPRS: 1 = harmless ($E < 30\%$), 2 = slightly harmful ($30 \leq E < 79\%$), 3 = moderately harmful ($80 \leq E < 99\%$), and 4 = harmful ($E \geq 99\%$). [4] Means followed by the same letter in a column do not differ significantly (GLM with quasi-Poisson distribution, followed by post hoc Tukey test; $p < 0.05$). [5] Means followed by the same letter in a column do not differ significantly (GLM with quasi-binomial distribution, followed by post hoc Tukey test; $p < 0.05$).

chlorpyrifos and malathion were more toxic than phosmet to *C. cubana* may be related to the formulations because chlorpyrifos and malathion are formulated as emulsifiable concentrates (EC), which contain an organic solvent, while phosmet is a wettable powder (WP) formulation containing wetting agents and a surfactant (dispersant).

The neonicotinoid thiamethoxam showed lethal and sublethal effects and it was not possible to estimate the demographic effects because of the few number of couples formed. Imidacloprid SC and WG had lethal effect on first-instar larvae and adults of *C. cubana* and sublethal effects which were observed in the life table parameters (Table 3). The lethal and sublethal effects of the direct contact of imidacloprid have already been watched out on other species of lacewings (Bueno & Freitas 2001, Rezaei *et al* 2007) and on others non-target insects like bees and ladybugs (Xiao *et al* 2016, Chen *et al* 2017). The neonicotinoids also have a systemic action in plants and they can cause side effects on the lacewings since these insects feed on floral nectar as observed for Gontijo *et al* 2014. Therefore, the use of neonicotinoids should be done in a rational way to avoid those side effects on the non-target insects.

The insecticides lambda-cyhalothrin + chlorantraniliprole and lambda-cyhalothrin + thiamethoxam were toxic to first-instar larvae and adults of *C. cubana*. Both insecticides had high lethal effect on larval and adult bioassay. Insects that remained alive in the lambda-cyhalothrin + thiamethoxam in the larval bioassay had sublethal effects like reduction of viability and increase in pupal duration and the emergency of males only. The isolated toxicity of each of these molecules has already been studied: lambda-cyhalothrin was toxic to larva and adults of lacewings (Sabry & El-Sayed 2011,

Table 3 Life table parameters of *Ceraeochrysa cubana* when first-instar larvae were sprayed with insecticides.

Treatments	Concentration (g a.i. L^{-1})	Population parameters[1]				
		R_o	r_m	*T* (days)	Td (days)	λ
Esfenvalerate	0.125	81.93bc	0.11ab	39.94c	6.28b	1.12ab
Imidacloprid SC	0.04	36.50c	0.08bc	45.80bc	8.83ab	1.08c
Imidacloprid WG	0.032	94.78b	0.08bc	53.48a	8.14ab	1.09c
Phosmet	0.25	72.40bc	0.06c	55.08a	8.92a	1.08c
Pyriproxyfen	0.0625	164.29b	0.11b	47.43b	6.44b	1.11b
Control	–	280.92a	0.12a	46.97bc	5.77c	1.13a

[1] The net reproductive rate (R_o), intrinsic rate of natural increase (r_m), finite rate of increase (λ), mean generation time (*T*), and population doubling time (*Td*). Means followed by the same letter in columns do not differ statistically by Student's *t* test ($p < 0.05$).

Table 4 Lethal and sublethal effects of pesticides sprayed on adults of *Ceraeochrysa cubana*.

Treatments	Concentration (g a.i. L^{-1})	Survival (%)[4]	Mean daily fecundity	Fertility (%)	MC[1]	*E* (%)[2]	Class (IOBC)[3]
Azadirachtin	0.03	12.50 ± 6.91b	–	–	85.71	–	4
Chlorpyrifos	0.72	0.00c	–	–	100	–	4
Esfenvalerate	0.125	8.33 ± 4.17b	–	–	90.48	–	4
Imidacloprid SC	0.04	12.50 ± 6.91b	–	–	85.71	–	4
Imidacloprid WG	0.032	16.67 ± 5.89b	9.10	88.00	80.95	86.04	3
Lambda-cyhalothrin + chlorantraniliprole	0.015 + 0.03	0.00c	–	–	100	–	4
Lambda-cyhalothrin + thiamethoxam	0.016 + 0.021	0.00c	–	–	100	–	4
Malathion	1.5	0.00c	–	–	100	–	4
Phosmet	0.25	12.50 ± 6.91b	8.10	90.00	85.71	90.47	3
Pyriproxyfen	0.0625	16.67 ± 5.89b	9.45	88.00	80.95	85.51	3
Thiamethoxam	0.025	0.00c	–	–	100	–	4
Control	–	87.50 ± 3.61a	12.24	89.30	–	–	–

[1] Corrected mortality by Abbott's formula (1925); [2] Total effect: $E = 100 - (100 - Mc) \times Er$; [3] Toxicity class proposed by IOBC/WPRS: 1 = harmless ($E < 30\%$), 2 = slightly harmful ($30 \leq E < 79\%$), 3 = moderately harmful ($80 \leq E < 99\%$), and 4 = harmful ($E \geq 99\%$). [4] Means followed by the same letter in a column do not differ significantly (GLM with quasi-binomial distribution, followed by post hoc Tukey test; $p < 0.05$).

Amarasekare & Shearer 2013), while chlorantraniliprole is selective to some lacewing species (Dinter *et al* 2008, Zotti *et al* 2013, Sabry *et al* 2014) and is highly toxic to other lacewing species (Amarasekare & Shearer 2013, Gontijo *et al* 2014). Thiamethoxam, as observed in this study, also has side effects on the lacewings (Gontijo *et al* 2014, Rugno *et al* 2015), although lambda-cyhalothrin and chlorantraniliprole or thiamethoxam in the same product may be more toxic to the lacewings due to the action at two different sites in the predator.

Azadirachtin is a botanic pesticide from the Indian neem tree and it is widely used in different crops in the world like alternatives to synthetic chemical insecticides for pest management (Zehnder *et al* 2007, IBD 2014). However, the results of our study proved sublethal effects of azadirachtin when sprayed on first-instar larvae of *C. cubana*, reducing the pupal survival and increasing the number of emerged males. Vogt *et al* (1998) listed possible causes of lacewing pupae mortality with azadirachtin: feeding reduction, alterations in muscle and tegument which affect the mobility, and production of ecdysteroids. One of the hypotheses about the high emergence of males is that females may be more sensitive in the immature stage. In adults, azadirachtin decreased the survival, longevity, and fecundity of females and was also considered harmful to this development stage. In addition to the observed effects in this study, azadirachtin may also have a repellent effect on lacewings (Cordeiro *et al* 2010), effects on the larval metamorphosis (Qi *et al* 2001), and effects on fertility (Medina *et al* 2003a, Medina *et al* 2004). Therefore, azadirachtin should be used with caution, because even though it is considered a biopesticide, its real effects on natural enemies are not still completely known. However, our study showed some side effects.

The pyrethroid esfenvalerate had lethal effect on adults of *C. cubana* and this reduction of survivorship was also observed in the study with the lacewing *C. externa* that showed sensibility to esfenvalerate (Ulhôa *et al* 2002). In the larva bioassay, negative effects of esfenvalerate were only observed in the life table parameters, showing an interference in the population growth. Sublethal effects were already observed in semi-field conditions when Carvalho *et al* (2003) studied the effects of esfenvalerate on *C. externa*, showing that these insecticides could compromise the performance of lacewings in greenhouses resulting in undesirable levels of pest control.

The juvenile hormone analogue insecticide, pyriproxyfen, is selective for some non-target insects, and for this reason, it is recommended to use in IPM programs (Medina *et al* 2002, 2003a, Godoy *et al* 2010, Moscardini *et al* 2013). However, *C. cubana* proved to be sensitive when pyriproxyfen was sprayed on larva and adult stages. This insecticide reduced survival in the adult bioassay and it had side effects that it reduced the intrinsic rate of natural increase. Sublethal effects of this insecticide on lacewings were already mentioned by Medina *et al* (2003b) that observed reduction in fertility on lacewing females of *C. carnea*. Pyriproxyfen has demonstrated sublethal effects on lacewings and the life table analyses became into a good tool to evaluate the side effects of this insecticide as we could remark in this study.

In conclusion, none of these 11 insecticides used in *D. citri* management programs was harmless to first-instar larvae and adults of *C. cubana* under laboratory conditions. In addition to the lethal effect, it is also important to highlight the sublethal effects that these insecticides caused which affected the life table parameters of lacewings and, consequently, the population dynamics when exposed to these insecticides.

These results will be useful for future research in semi-field and field conditions, as well as for other species of lacewings.

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