

# Impact of insect growth regulators on the predator Ceraeochrysa cincta (Schneider) (Neuroptera: Chrysopidae)

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Abstract The generalist predator Ceraeochrysa cincta (Schneider) (Neuroptera: Chrysopidae) is an important biological control agent of several arthropod pests in different agroecosystems. This study assessed the lethal and sublethal effects of six insect growth regulators sprayed on first-instar larvae of C. cincta. Lufenuron and diflubenzuron were highly harmful to first-instar larvae of C. cincta, causing 100 % of mortality before they reached the second instar. Buprofezin caused  $\sim$  25 % mortality of the larvae and considerably reduced the fecundity and longevity of the insects, but substantially increased the proportion of females in the surviving population of C. cincta. Methoxyfenozide and tebufenozide did not affect the duration and survival of the immature stages, but methoxyfenozide significantly reduced the fecundity and longevity of the insects. Pyriproxyfen reduced the survival of the larval stage by 19.5 %, but did not affect the development, survival and reproduction of the surviving individuals. Based on reduction coefficient, the insecticides diflubenzuron and lufenuron were considered harmful to C. cincta, whereas buprofezin and methoxyfenozide were slightly harmful and tebufenozide and pyriproxyfen were harmless. The estimation of lifetable parameters indicated that buprofezin and methoxyfenozide significantly reduced the  $R_o$ , r and  $\lambda$  of C. cincta, whereas pyriproxyfen and tebufenozide caused no adverse effect on population parameters, indicating that these insecticides could be suitable for use in pest management

 $\boxtimes$  Gabriel Rodrigo Rugno gabrielrugno@gmail.com programs towards the conservation and population increase of the predator in agroecosystems. However, more studies should be conducted to evaluate the compatibility of these insecticides with the predator C. cincta under semi-field and field conditions.

Keywords Lacewings - Insecticides - Lethal and sublethal effects - Life-table

## Introduction

Worldwide, pesticides play an important role in regulating arthropodpest populations that affect agricultural production systems, protecting plants against direct and indirect damage caused by herbivores (Adhikari et al. [2004;](#page-7-0) Marimuthu et al. [2013;](#page-9-0) Rugno et al. [2015;](#page-9-0) Santos et al. [2015](#page-9-0)). However, although pesticides reduce populations and mitigate the impacts of arthropod pests, they also affect ecological processes, causing mortality of natural enemies, selection of resistant pest populations to the main active ingredients, resurgence of pests targeted for control, and outbreaks of secondary pests (Desneux et al. [2007;](#page-8-0) Cutler et al. [2009;](#page-8-0) Szczepaniec et al. [2011](#page-9-0); Lu et al. [2012](#page-8-0); Biondi et al. [2012a;](#page-7-0) Cordeiro et al. [2013;](#page-8-0) Guedes and Cutler [2014](#page-8-0); Zhan et al. [2015\)](#page-9-0), increasing production costs and reducing the technical efficacy and environmental sustainability of the system (Ribeiro et al. [2014\)](#page-9-0). Given these problems and the growing consumer demand for healthier food produced in more sustainable environments, new chemical groups of insecticides with different mechanisms of action have appeared on the market and have been used in integrated pest management (IPM) programs as alternatives to neurotoxic insecticides, in order to reduce impacts on nontarget organisms.

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<span id="page-1-0"></span>Insect growth regulators have been widely used in the control of arthropod pests in many crops, due to their high efficacy, rapid degradation of residues in the environment, and low toxicity to higher animals (Dhadialla et al. [1998](#page-8-0); Arthur et al. [2009](#page-7-0); Tiwari et al. [2012\)](#page-9-0). These products act by inhibiting chitin biosynthesis or as analogues of juvenile and ecdysteroid hormones, which are involved in ecdysis in insects (Meola and Mayer [1980](#page-9-0); Arthur et al. [2009](#page-7-0)). Despite their high toxicity to arthropod pests, these insecticides have been considered compatible with biologicalcontrol agents present in agricultural ecosystems (Liu and Chen [2000\)](#page-8-0). However, studies have shown that exposure to this class of insecticide may cause high mortality of different developmental stages of predators and parasitoids (Hattingh and Tate [1995](#page-8-0); Magagula and Samways [2000](#page-8-0); Yu et al. [2014\)](#page-9-0), while reducing the development of the immature stage (Liu and Chen [2000](#page-8-0)), reproduction (Mendel et al. [1994](#page-9-0)) and longevity of insects (Liu and Stansly [2004\)](#page-8-0), and reduce the biocontrol services provided by natural enemies (Biondi et al. [2015](#page-7-0)), which may compromise IPM programs that use natural biological control (Castro et al. [2012\)](#page-8-0) as a strategy to reduce the infestation levels of arthropod pests and economic damage to crops.

Among the biological agents that act as natural controls of arthropod pests, lacewings (Neuroptera: Chrysopidae) are one of the most important, due to their high biotic potential, voracity and ecological plasticity (Tauber et al. [2000;](#page-9-0) Pappas et al. [2011](#page-9-0); Khuhro et al. [2014\)](#page-8-0). In Neotropical agroecosystems, the lacewing Ceraeochrysa cincta (Schneider) is one of the most important generalist predators found in several crops, including citrus, tree plantations and banana (López-Arroyo et al. [1999;](#page-8-0) Tauber et al. [2000\)](#page-9-0). C. cincta is present in North, Central and South America (Ramírez-Delgado et al. [2007\)](#page-9-0) and its larvae are associated with populations of aphids, lace bugs, scales, whiteflies, psyllids and mites that cause serious crop damage (Tauber et al. [2000](#page-9-0)). In laboratory conditions, Cardoso et al. [\(2007](#page-8-0)) reported that each larva of C. cincta consumes 6275 nymphs or 129 adults of Leptopharsa heveae Drake and Poor (Hemiptera: Tingidae); Lopez and Freitas ([1996\)](#page-8-0) observed that larvae were able to predate 139 aphids [Rhodobium porosum (Sanderson) (Hemiptera: Aphididae)] during their development in rose-production systems. In addition, C. cincta can be easily multiplied in the laboratory, which allows the establishment of massrearing facilities, and inundative releases in crop areas to reduce arthropod pest populations (Tauber and De León [2001\)](#page-9-0), increasing the potential of this biological-control agent in IPM programs (López-Arroyo et al. [1999\)](#page-8-0).

Despite the potential of C. cincta as a biological-control agent of arthropod pests, no studies have examined the impacts of insect growth regulators on this predator species. This study evaluated the effects of six insect growth regulators on the development and reproduction of C. cincta. Based on these effects, population parameters were estimated to determine the impacts on and the compatibility of these products with the predator. This information will be important for the development of management strategies aiming to conserve and/or increase population levels of C. cincta, to ensure the success of biological control in IPM programs.

## Materials and methods

## Insects

The population was established from C. cincta specimens collected on citrus plants managed without pesticide application in the preceding 6 months in Piracicaba, São Paulo, Brazil. The species was confirmed by Dr. Francisco Sosa, Universidad Centroccidental ''Lisandro Alvarado'', Barquisimetro, Venezuela. The specimens were transferred to rearing cages made of polyvinyl chloride (PVC), 10 cm in diameter  $\times$  20 cm high with one end placed on an acrylic dish (15 cm in diameter) containing a filter-paper disc in the base (15 cm in diameter); the other end was closed with a tulle screen (100  $\mu$ m). The adults were fed with a diet based on brewer's yeast and honey (1:1, v:v) as proposed by Godoy et al. [\(2004](#page-8-0)). The food was made available to the insects by means of cotton discs placed on the cages. To obtain eggs, the cages were coated with white paper. The paper was replaced every 24 h and the eggs transferred to plastic pots of  $1500 \text{ cm}^3$ , with an opening  $\sim$  7.5 cm in diameter ( $\sim$  41.2 cm<sup>2</sup>) sealed with voile fabric. The larvae obtained were kept in the same plastic pots until the adults emerged. During this period, the larvae were fed ad libitum with eggs of Ephestia kuehniella (Zeller) (Lepidoptera: Pyralidae) sterilized under a germicidal lamp (UV) as described by Stein and Parra ([1987\)](#page-9-0). To perform the bioassays, fifth-generation larvae of C. cincta maintained under laboratory conditions were used. The rearing of C. cincta and the bioassays were performed in a climate-controlled room with temperature  $25 \pm 2$  °C, relative humidity (RH)  $70 \pm 10\%$  and 14L:10D h photoperiod.

## Insecticides

Six commercial insecticides were evaluated on C. cincta, representing the main active ingredients belonging to the insect growth regulators group recommended by the Ministry of Agriculture, Livestock and Supply [MAPA (Agrofit [2015](#page-7-0))] for control of the mite Phyllocoptruta oleivora (Ashmead) (Prostigmata: Eriophyidae), scales [Praelongorthezia praelonga (Douglas) (Hemiptera: Diaspididae),

Pulvinaria flavescens Brèthes (Hemiptera: Coccidae), Selenaspidus articulatus (Morgan) (Hemiptera: Diaspididae), Parlatoria cinerea Doane and Hadden (Hemiptera: Diaspididae)], citrus leafminer [Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae)], Asian citrus psyllid [Diaphorina citri Kuwayama (Hemiptera: Liviidae)], and Ecdytolopha aurantianum Lima (Lepidoptera: Tortricidae) in Brazilian citrus orchards. The insecticides and concentrations (g a.i.  $L^{-1}$ ) evaluated were: diflubenzuron 0.12 (Micromite 24 SC, Chemtura Indústria Química do Brasil Ltda.), lufenuron 0.25 (Match 5 EC, Syngenta Crop Protection Ltda.), buprofezin 0.50 (Applaud 25 SP, Arysta Lifescience of Brazil Chemical Industry and Agriculture), methoxyfenozide 0.75 (Intrepid 24 SC, Dow Agroscience Industrial Ltda.), tebufenozide 0.48 (Mimic 24 SC, Dow Agroscience) and pyriproxyfen 0.20 (Tiger 10 EC, Syngenta Crop Protection Ltda.). All four companies are located in São Paulo, SP, Brazil.

#### Bioassay

To assess the lethal and sublethal effects of these insecticides, 40 first-instar larvae  $(<24$  h old) were used for each treatment. For this purpose, first-instar larvae were anesthetized by 10 s and sprayed with 2 mL of solution in a Potter tower (Burkard Scientific Co., Uxbridge, UK), adjusted to a pressure of 68.6 kPa, resulting in a deposit of  $1.8 \pm 0.1$  mg cm<sup>-2</sup> of fresh residues, which is consistent with the criteria established by the Pesticides and Beneficial Organisms working group of the International Organization for Biological Control of Noxious Animals and Plants/West Palaearctic Regional Section (IOBC/WPRS) for pesticide toxicity studies on natural enemies (Hassan et al. [1994](#page-8-0)). Deionized water was used as a control treatment. Immediately after the treatment were applied, the larvae were placed in individual flat-bottom glass tubes  $(8.5 \times 2.5 \text{ cm}^2 \text{ in diameter})$ , which were sealed with PVC film and kept in a climate-controlled room. During the bioassay evaluation period, the larvae were fed ad libitum with sterilized E. kuehniella eggs (Stein and Parra [1987](#page-9-0)). For each treatment, 40 repetitions were used. The bioassay was repeated thrice over time using a fully randomized design.

Mortality and duration of the larval stage were assessed daily until the pupae were obtained. The pupae were kept in the same tubes used for larval development until the adults emerged. The viability of the pupal stage was determined based on the number of emerged adults. Adults were initially separated by sex, and then couples were formed and transferred to PVC cages (15 cm in diameter  $\times$  10 cm high) as described in "Insects" section, to evaluate the pre-oviposition and oviposition periods, fecundity and fertility of females, percentage of deformed eggs (number of eggs dehydrated and absence of pedicel in relation to control treatment) and longevity of females and males. During this period, the adults were fed with a mixture of brewer's yeast and honey (1:1, v:v) available in cotton discs on the cages. Treatments that provided fewer than seven couples were not used in further analyses due to the small number of surviving insects (repetitions). The evaluations were performed every 24 h until all adults died. Female fertility was calculated based on the number of larvae hatched from 100 eggs, due to the smaller number of eggs laid in first oviposition  $(n < 4)$ , it was standardized the collection from the second oviposition of each female. For this, eggs of each female were removed from the cages and placed in individual compartments of Elisa plates (EasyPath Ltda., São Paulo, SP, Brazil) as described by Godoy et al. [\(2004](#page-8-0)). The plates were sealed with transparent PVC film and kept in a climate-controlled room. The number of hatched larvae in each treatment was evaluated 12 d after the transfer to the plates.

#### Data analysis

## Effect of insecticides on the development and reproduction of C. cincta

Generalized linear models (Nelder and Wedderburn [1972\)](#page-9-0) with quasi-binomial and quasi-Poisson distributions were used to analyze mortality/survival proportion data (larvae and pupae) and counts (number of eggs and duration of larval and pupal stages), respectively. The quality adjustment was determined through a half-normal graph with a simulation envelope (Hinde and Demétrio [1998](#page-8-0)). In cases of significant differences between treatments, multiple comparisons with the Tukey test ( $p < 0.05$ ) for balanced data and Tukey–Kramer test ( $p < 0.05$ ) for unbalanced data were made through the "glht" and "multcomp" and " $DTK$ " packages, respectively, with adjusted  $p$  values. All analyses were performed using the statistical software  $"R"$ , version 3.1.3 (R Development Core Team [2015\)](#page-9-0).

## Toxicity class

Based on the lethal (mortality of larvae and pupae) and sublethal effects (fecundity and fertility), the reduction coefficient  $(E_x)$  was calculated for each treatment using the formula  $E_x = 100 - (100 - M_c) \times E_r$  proposed by Biondi et al. ([2012b\)](#page-7-0), where:  $M_c$  = corrected mortality calculated with the formula of Abbott ([1925\)](#page-7-0), and  $E_r =$  effect on reproduction, calculated as:  $E_r = R_1 \times R_2$  where:  $R_1 =$ ratio of the number of eggs laid by females from first-instar larvae treated with insecticides and control, and  $R_2$  = ratio of the number of hatched larvae for females from firstinstar larvae treated with insecticides and control. Based on reduction coefficient, the insecticides were classified according to the toxicity scale proposed by IOBC/WPRS, which include four categories: I, harmless ( $E_x$  < 30 %); II, slightly harmful (30 %  $\le E_x \le 79$  %); III, moderately harmful (80 %  $\le E_x \le 99$  %) and IV, harmful  $(E_x > 99 \%)$  as described by Biondi et al. [\(2012b](#page-7-0)).

## Estimation of the demographic growth parameters

Based on the data for duration and survival of the immature stages, pre-oviposition period, fecundity and fertility of females, and longevity of females and males of C. cincta, life-table parameters were estimated for each treatment. Life tables were constructed based on data for all individuals tested (including females, males and individuals that died during the immature stage development, as proposed by Chi ([1988\)](#page-8-0). The original data for all individuals were analyzed according to the theoretical model proposed by Chi and Liu ([1985\)](#page-8-0), using the TWOSEXMSChart program (Chi [2014](#page-8-0)). For each treatment were estimated:The net reproductive rate  $(R<sub>o</sub>)$ :

$$
R_o = \sum_{x=0}^{\infty} l_x m_x
$$

The intrinsic rate of increase  $(r)$ :

$$
\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1
$$

The mean generation time  $(T)$ :

$$
T=\ln R_o/r
$$

and the finite rate of increase  $(\lambda)$ :

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\lambda = e^r
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The intrinsic growth rate was estimated by the Euler– Lotka formula (Goodman [1982\)](#page-8-0). The means and standard errors of each parameter were estimated by the bootstrap method, following the procedure of Huang and Chi [\(2012](#page-8-0)). During the bootstrap procedure, the data for each population parameter were resampled 40,000 times. The means of each treatment were compared by paired bootstrap test  $(p<0.05)$  (Efron and Tibshirani [1993](#page-8-0)).

# Results

The spraying of insect growth-regulator insecticides on first-instar larvae of C. cincta showed that lufenuron and diflubenzuron were highly harmful to the predator, causing 100 % mortality of larvae before they reached the second instar, differing significantly from the other treatments (Table [1](#page-4-0)). Buprofezin and pyriproxyfen reduced C. cincta larval survival to 65.2 and 70.5 %, respectively, whereas methoxyfenozide and tebufenozide did not significantly affect the survival of larvae, which was similar to the control (Table [1](#page-4-0)).

The duration of the larval stage and the duration and survival of the pupal stage were not affected by pyriproxyfen, buprofezin, methoxyfenozide and tebufenozide (Table [1\)](#page-4-0). The pre-oviposition period of females was not affected by any of the insecticides (Table [2](#page-4-0)). However, the number of eggs laid by females (fecundity) was significantly lower in females from first-instar larvae treated with buprofezin and methoxyfenozide, compared to the other treatments (Table [2\)](#page-4-0). No significant effect was observed on the oviposition period and fertility of females from firstinstar larvae treated with insect growth-regulator insecticides (Table [2](#page-4-0)). The higher percentage of deformed eggs (dehydrated and absence of pedicel) was observed in females from first-instar larvae treated with tebufenozide and pyriproxyfen compared to females from first-instar larvae treated with buprofezin, methoxyfenozide and the control treatment (Table [2\)](#page-4-0). The longevity of females and males was reduced more in the treatments with buprofezin and methoxyfenozide than in the other treatments (Table [2\)](#page-4-0).

Based on mortality of larvae and pupae and sublethal effects (fecundity and fertility) were used to estimate the reduction coefficient for each insecticide. Our results showed that diflubenzuron and lufenuron were harmful  $(E_x > 99 \%$ , class IV) to *C. cincta*, whereas buprofezin and methoxyfenozide were slightly harmful (30  $\le E_x \le 79$  %, class II) and pyriproxyfen and tebufenozide were harmless  $(E_x < 30 \%$ , class I) (Table [3\)](#page-5-0).

The life-table parameters were estimated based on the data for duration and survival of the immature stages, preoviposition and oviposition periods, fecundity, fertility, and longevity of males and females obtained in the different treatments (except for diflubenzuron and lufenuron, which caused 100 % larval mortality). Methoxyfenozide and buprofezin considerably reduced the net reproductive rate  $(R<sub>o</sub>)$ , intrinsic rate of increase  $(r)$  and finite rate of increase  $(\lambda)$  of *C. cincta* (Table [4](#page-5-0)). On the other hand, pyriproxyfen and tebufenozide did not affect the  $R_0$ , r, and  $\lambda$  and were similar to the control (Table [4\)](#page-5-0). Spraying of methoxyfenozide, buprofezin, pyriproxyfen, and tebufenozide on first-instar larvae of C. cincta did not significantly alter the mean generation time  $(T)$  (Table [4\)](#page-5-0).

## Discussion

The lethal and sublethal effects of six insect growth-regulator insecticides on first-instar larvae of C. cincta were evaluated. Our results showed that the toxicity levels of the

<span id="page-4-0"></span>Table 1 Duration and survival (mean  $\pm$  SE) of larvae and pupae of Ceraeochrysa cincta when first-instar larvae were treated topically with insect growth-regulator insecticides



<sup>a</sup> Means followed by the same letter in a column do not differ significantly (GLM with quasi-Poisson distribution, followed by post hoc Tukey test;  $p < 0.05$ )

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

**Table 2** Sublethal effects of pesticides on biological parameters (mean  $\pm$  SE) of the adult stage of *Ceraeochrysa cincta* when first-instar larvae were treated topically with insect growth-regulator insecticides

Treatment	Pre- oviposition period <sup>a</sup> (d)	Oviposition period <sup>a</sup> (d)	Fecundity <sup>a</sup> (mean number of eggs $female^{-1}$ )	Fertility <sup>b</sup> (number of hatched larvae) $(\%)$	Deformed eggs <sup>a</sup> (dehydrated and absence of pedicel) $(\%)$	Longevity <sup>a</sup> (d)	
						Female	Male
Control (deionized) water)	$14.8 \pm 0.72$ a	$45.0 \pm 0.63$ a	$228.2 \pm 28.52$ a	$90.6 \pm 2.43$ a	$0.1 \pm 0.02$ d	58.4 $\pm$ 3.52 a	49.1 $\pm$ 2.47 a
Buprofezin $(0.50 \text{ g L}^{-1})$	$13.2 \pm 1.74$ a	$43.6 \pm 1.68$ a	$101.6 \pm 19.59 b$	$85.4 \pm 2.36$ a	$3.5 \pm 0.21$ b	$35.5 \pm 3.06$ b	$35.2 \pm 4.52 b$
Methoxyfenozide $(0.75 \text{ g L}^{-1})$	$13.7 \pm 1.55$ a	$44.5 \pm 1.29$ a	$106.8 \pm 21.35 b$	$86.5 \pm 3.10$ a	$1.3 \pm 0.09$ c	$38.5 \pm 5.23 b$	$33.6 \pm 1.79 b$
Pyriproxyfen $(0.20 \text{ g L}^{-1})$	$16.3 \pm 1.89$ a	$47.1 \pm 1.65$ a	$187.4 \pm 27.92$ a	$89.2 \pm 3.30$ a	$4.5 \pm 0.12$ a	$52.6 \pm 2.86$ a	$45.7 \pm 2.74$ a
Tebufenozide $(0.48 \text{ g L}^{-1})$	$18.8 \pm 1.64$ a	$47.6 \pm 1.65$ a	$203.4 \pm 30.01$ a	$85.0 \pm 1.73$ a	$4.4 \pm 0.17$ a	59.1 $\pm$ 2.20 a	$53.6 \pm 5.04$ a
	$F = 1.972$	$F = 1.405$	$F = 3.305$	$F = 1.009$	$F = 3.449$	$F = 3.610$	$F = 3.002$
	$d.f. = 4, 55$	$d.f. = 4, 55$	$d.f. = 4, 55$	$d.f. = 4, 15$	$d.f. = 4, 55$	$d.f. = 4, 55$	$d.f. = 4, 55$
	$p = 0.112$	$p = 0.244$	$p = 0.041$	$p = 0.634$	$p = 0.041$	$p = 0.011$	$p = 0.034$

<sup>a</sup> Means followed by the same letter in a column do not differ significantly (GLM with quasi-Poisson distribution, followed by post hoc Tukey test;  $p < 0.05$ )

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

insecticides were dependent on the chemical group used in the bioassays. The application of chitin-biosynthesis inhibitors type 0 (lufenuron and diflubenzuron) were highly harmful (class IV) to first-instar larvae of C. cincta, causing 100 % mortality before the second instar. These results are similar to those observed in first- and third-instar larvae of Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae) exposed to lufenuron (Hussain et al. [2012;](#page-8-0) Mohammadi et al. [2014\)](#page-9-0) and diflubenzuron (Medina et al. [2003a\)](#page-9-0). A high mortality rate was also reported by Zotti et al. ([2012\)](#page-9-0) in third-instar larvae of Chrysoperla externa (Hagen) (Neuroptera: Chrysopidae) treated topically with lufenuron. Bueno and Freitas ([2004\)](#page-8-0) demonstrated that the application of lufenuron on eggs of C. externa significantly reduced the larval survival rate at the time of ecdysis, whereas Medina et al. ([2003a](#page-9-0)) found lower survival of

Treatment	Cumulative mortality of	Effect on reproduction $(E_r)$	Reduction	Toxicity	
	immature stage $(\%)$	Fecundity (mean number of eggs laid female <sup><math>-1</math></sup> ) ( $R_1$ )	Fertility $(\%)$ (percent of hatched larvae) $(R_2)$	coefficient $(E_x)^a$	categories <sup>b</sup>
Control (deionized water)	17.0	228.2	90.6		
Buprofezin $(0.50 \text{ g L}^{-1})$	35.0	101.6	85.4	65.8	П
Diflubenzuron $(0.12 \text{ g L}^{-1})$	100.0			100.0	IV
Lufenuron $(0.25 \text{ g L}^{-1})$	100.0			100.0	IV
Methoxyfenozide $(0.75 \text{ g L}^{-1})$	24.5	106.8	86.5	58.5	$\mathbf{I}$
Pyriproxyfen $(0.20 \text{ g L}^{-1})$	29.5	187.4	89.2	29.3	I
Tebufenozide $(0.48 \text{ g L}^{-1})$	26.2	203.4	85.0	24.5	I

<span id="page-5-0"></span>Table 3 Reduction coefficient of insect growth-regulator insecticides applied topically on first-instar larvae of Ceraeochrysa cincta

<sup>a</sup> Reduction coefficient of insecticides calculated by formula proposed by Biondi et al.  $(2012b)$ 

<sup>b</sup> Toxicity categories proposed by the IOBC/WPRS for pesticide selectivity studies on natural enemies: I, harmless ( $E_x < 30 \%$ ); II, slightly harmful (30 %  $\le E_x \le 79$  %); III, moderately harmful (80 %  $\le E_x \le 99$  %) and IV, harmful ( $E_x > 99$  %) as described by Biondi et al. [\(2012b](#page-7-0))

Table 4 Estimates of life-table parameters of Ceraeochrysa cincta when first-instar larvae were treated topically with insect growth-regulator insecticides

Treatment	Population parameter (mean $\pm$ SE) <sup>a</sup>						
	Net reproduction rate $(Ro)$ (female female <sup><math>-1</math></sup> )	Mean generation time $(T)$ (d)	Intrinsic rate of increase $(r)$ (female female <sup>-1</sup> d <sup>-1</sup> )	Finite rate of increase $(\lambda)$ (female female <sup><math>-1</math></sup> d <sup><math>-1</math></sup> )			
Control (deionized water)	$80.03 \pm 14.63$ a	57.66 $\pm$ 1.49 ab	$0.076 \pm 0.006$ a	$1.08 \pm 0.006$ a			
Buprofezin $(0.50 \text{ g L}^{-1})$	$27.91 \pm 11.01$ b	52.84 $\pm$ 2.60 b	$0.063 \pm 0.008$ b	$1.06 \pm 0.009$ b			
Methoxyfenozide $(0.75 \text{ g L}^{-1})$	$31.97 \pm 14.97$ b	$57.75 \pm 3.01$ ab	$0.060 \pm 0.009$ b	$1.06 \pm 0.010$ b			
Pyriproxyfen $(0.20 \text{ g L}^{-1})$	$61.05 \pm 12.88$ ab	57.11 $\pm$ 2.13 ab	$0.072 \pm 0.008$ ab	$1.07 \pm 0.008$ ab			
Tebufenozide $(0.48 \text{ g L}^{-1})$	$51.24 \pm 11.43$ ab	$60.56 \pm 1.58$ a	$0.065 \pm 0.007$ ab	$1.07 \pm 0.007$ ab			

<sup>a</sup> Means followed by the same letter on a line do not differ significantly by the bootstrap paired test ( $p < 0.05$ )

pupae when third-instar larvae were treated with this insecticide, demonstrating that lufenuron can also affect the survival of subsequent stages of those exposed to the insecticides. The high mortality of first-instar larvae treated with diflubenzuron and lufenuron observed in this study is attributed to inactivation of the enzyme chitin synthase, responsible for chitin biosynthesis in the new integument of insects during ecdysis (Desneux et al. [2007](#page-8-0); Castro et al. [2012\)](#page-8-0), and to the low rate of excretion of the products by insects (Medina et al. [2002](#page-9-0)).

Although not evaluated in this study (due to high mortality of first-instar larvae), previous studies have shown that diflubenzuron and lufenuron can also affect reproduction when applied to adult lacewings. Medina et al. [\(2002](#page-9-0)) demonstrated that topical application of diflubenzuron on C. carnea adults completely inhibited the larvae hatching, and Godoy et al. [\(2004](#page-8-0)) found that spraying of lufenuron on adults of C. externa considerably reduced the fecundity and fertility of females. According to the authors, these effects are associated with malformation of the

reproductive cells in females (oogenesis) and/or males (spermatogenesis) in insects exposed to these insecticides. Diflubenzuron can also reduce the deposition of yolk in oocytes and prevent the formation of layers of follicular epithelial and cytoplasmatic cells in females, and cause disruption of spermatocytes in males (Agüero et al.  $2014$ ). These results indicate that excessive use of diflubenzuron and lufenuron in agroecosystems can substantially reduce the population levels of predators and compromise the natural biological control exerted by lacewings, specifically C. cincta.

On the other hand, the spraying of chitin biosynthesis inhibitor type 1 (buprofezin, 0.50  $g L^{-1}$ ) on first-instar larvae of C. cincta caused low acute toxicity ( $\sim$  27 % mortality in relation to the control) for the immature stage of the predator. Similar acute toxicity was observed in larvae of Chrysoperla rufilabris (Burmeister) (Neuroptera: Chrysopidae) (Nasreen et al. [2005;](#page-9-0) Sabry and El-Sayed [2011](#page-9-0)) and C. carnea (Liu and Chen [2000\)](#page-8-0) treated with different concentrations of buprofezin. However, James [\(2004](#page-8-0)) reported that buprofezin (0.58  $\rm g\ L^{-1}$ ) caused high mortality of *Harmonia* axyridis (Pallas) (Coleoptera: Coccinellidae) larvae and Geocoris pallens Stål and Geocoris punctipes (Say) (Hemiptera: Geocoridae) nymphs exposed to product resi-dues. Likewise, Cabral et al. ([2008\)](#page-8-0) reported  $\sim 67$  % mortality of larvae Coccinella undecimpunctata L. (Coleoptera: Coccinellidae) exposed to buprofezin (0.125  $g L^{-1}$ ). These results indicate that lacewing larvae are more tolerant to buprofezin than are coccinellid and hemipteran predators. This concord with the findings of Grafton-Cardwell et al. [\(2005](#page-8-0)), who reported high activity of buprofezin for larvae of beetle and nymphs of hemipteran pests, and with Vivek et al. [\(2012](#page-9-0)), who documented a small effect of this insecticide on lacewing larvae.

Our results demonstrated that females from first-instar larvae treated with buprofezin produced fewer eggs compared to the control treatment. Moreover, buprofezin reduced the longevity of C. cincta and increased the proportion of deformed eggs. The lower number of eggs laid by the females may be associated with the shorter oviposition period (low longevity); inhibition of prostaglandin  $E_2$ biosynthesis, one of the main components associated with oviposition (Moreno and Nakano [2002\)](#page-9-0); and changes in oviposition behavior of the predator, as found in females of Encarsia formosa (Gahan) (Hymenoptera: Aphelinidae) (Gholamzadeh et al. [2012](#page-8-0)) and other natural enemies (Desneux et al. [2007\)](#page-8-0). Likewise, the reduced longevity of C. cincta could be attributed to increase in 20-hydroxyecdysone hormone levels after apolysis (De Cock and Degheele [1993](#page-8-0)), causing a hormonal imbalance in adult insects, which affects survival (Moreno and Nakano [2002](#page-9-0)).

Our results also showed that the toxicity levels of buprofezin were lower than those observed for diflubenzuron and lufenuron. Although the additional action mechanisms of these insecticides are not completely known, the low penetration capacity of buprofezin in the integument and its excretion by insects may be some of the causes for the lower toxicity of buprofezin to C. cincta compared to diflubenzuron and lufenuron. Medina et al. [\(2003a\)](#page-9-0) found that the diflubenzuron penetration rate was 10–20 % 1 h after application of the product on C. carnea larvae. Diflubenzuron is also eliminated (excreted) more slowly, which may increase its toxicity and action period. Although the lower impact on survival and reproduction of C. cincta, buprofezin considerably reduces the net reproductive rate  $(R_o)$ , intrinsic rate of increase  $(r)$  and the finite rate of increase  $(\lambda)$  of the predator. Therefore, the use of this insecticide can compromise the biological control exerted by predator C. cincta in IPM programs.

The exposure of first-instar larvae to ecdysteroid receptor agonists indicated that methoxyfenozide and tebufenozide did not cause significant larval mortality of C. cincta. Similar results were obtained for larvae of C. externa (Ferreira et al. [2005;](#page-8-0) Rimoldi et al. [2008](#page-9-0)) and C. carnea (Ferreira et al. [2006\)](#page-8-0) treated with these insecticides. Although considered harmless to the immature stage, methoxyfenozide reduced the  $R_o$ , r and  $\lambda$  of C. cincta and was considered slightly harmful in the IOBC/WPRS classification. The reduction of population parameters is directly related to the reduction of the oviposition rate of females and the longevity of the insects. The action of methoxyfenozide on ecdysteroid receptors disrupts the reproductive process (vitellogenesis, production of mature eggs and promoting the growth of spermatocytes), reducing the reproductive rate of insects (Desneux et al. [2007](#page-8-0)). These results indicate that the use of methoxyfenozide in production systems can reduce population growth and the effectiveness of the predator in controlling arthropod pests. It should be emphasized, however, that this study was conducted in the laboratory, where insects are subjected to maximum contact with the insecticide. Probably, under field conditions, the sublethal effects of methoxyfenozide will be lower than those observed here. This hypothesis is supported by the findings of Colomer et al. [\(2011](#page-8-0)), who reported no significant effect on population levels of Orius laevigatus Fieber (Hemiptera: Anthocoridae) and Amblyseius swirskii (Athias-Henriot) (Acari: Phytoseiidae) after application of methoxyfenozide. In the present study, tebufenozide did not alter the survival, reproduction and population parameters of C. cincta and was considered harmless ( $E_x < 30$  %, class I) to the predator. Similarly, no adverse effects were observed on C. carnea (Medina et al. [2002](#page-9-0), [2003a,](#page-9-0) [b\)](#page-9-0) and Micromus tasmaniae Walker (Neuroptera: Hemerobiidae) (Rumpf et al. [1998](#page-9-0)) treated with tebufenozide. The safety of tebufenozide is attributed to its low rate of absorption and penetration in the insect

<span id="page-7-0"></span>integument, e.g.,  $\sim$  45 % 24 h after application of the product (Medina et al. [2003a\)](#page-9-0). Moreover, tebufenozide can occupy the binding site of ecdysone, preventing any deleterious effects of the product on lacewings (Zotti et al. [2012\)](#page-9-0). Our results indicated that tebufenozide can be used in IPM programs, because it did not affect the biological and population parameters of the predator C. cincta.

Similarly, the juvenile hormone analogue (pyriproxyfen) was considered harmless ( $E_x < 30$  %, class I) to C. cincta, although it slightly reduced the larval survival rate and slightly increased the proportion of deformed eggs. Pyriproxyfen was also judged to be safe for Ceraeochrysa cubana (Hagen) (Neuroptera: Chrysopidae) (Godoy et al. [2010\)](#page-8-0), C. externa (Godoy et al. [2010;](#page-8-0) Torres et al. [2013](#page-9-0)), and C. carnea (Medina et al. [2002](#page-9-0), [2003a,](#page-9-0) [b\)](#page-9-0). The low toxicity of pyriproxyfen is attributed to its rapid excretion by neuropterans. Medina et al. [\(2003a\)](#page-9-0) found that most of the insecticide was excreted 48 h after application. In addition to the relatively small lethal effect, pyriproxyfen did not affect the fecundity, fertility or longevity of C. cincta. Similarly, no significant effects on reproduction (fecundity and fertility) and on adult survival were observed for C. cubana (Godoy et al. [2004](#page-8-0)), C. externa (Medina et al. [2003b](#page-9-0); Godoy et al. [2004](#page-8-0); Torres et al. [2013\)](#page-9-0) and C. carnea (Medina et al. [2003a,](#page-9-0) [b\)](#page-9-0) exposed to pyriproxyfen. In addition to lacewings, pyriproxyfen was also considered safe for fifth-instar nymphs of O. laevigatus treated with this insecticide.

On the other hand, pyriproxyfen increased the proportion of deformed eggs in the surviving population. The deposition of inviable eggs and absence of pedicels in these eggs may be associated with hormonal alteration (especially juvenile hormone) caused by this insecticide. During the development of the immature stage, the juvenile hormone is responsible for maintaining the juvenile features of the insects, whereas in adults this hormone acts in the synthesis of vitellogenin, which is secreted into the hemolymph and used in the formation of oocytes (Guo et al. [2014](#page-8-0)). Thus, the application of pyriproxyfen can cause a hormonal imbalance that affects vitellogenesis, causing abnormalities in the formation, development and viability of eggs (Agüero et al.  $2014$ ). Although significant, these effects did not cause any significant alteration in  $R<sub>o</sub>$ ,  $r$  and  $\lambda$  of C. cincta, demonstrating that pyriproxyfen is selective and can be used in combination with the predator for population management of arthropod pests.

Although the insect growth-regulator insecticides are considered harmless to many species of beneficial arthropods, our results showed that the use of diflubenzuron, lufenuron, buprofezin and methoxyfenozide for the control of insect pests may adversely affect population levels of C. cincta and compromise the predator's performance as a biological control agent. However, more studies on the selectivity to C. cincta with the tested compound should be conducted using different development stages of the predator as well as other methods of exposure this insecticides. In addition, semi-field and field studies may also contribute to understand the impacts of these compounds on population levels of the predator. On the other hand, pyriproxyfen and tebufenozide did not affected the population parameters of C. *cincta*, suggesting that these insecticides would be highly suitable for use in pest management programs towards the conservation and population increase of the predator. The present study provides useful information for integrated pest-management programs that aim to combine chemical and biological control for suppression of arthropod pest populations.

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#### Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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