

RESEARCH ARTICLE

Toxicity assessment of four insecticides with different modes of action on pupae and adults of *Eriopis connexa* (Coleoptera: Coccinellidae), a relevant predator of the Neotropical Region

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Abstract Pesticides can be toxic to nontarget organisms including the natural enemies of agricultural pests, thus reducing the biodiversity of agroecosystems. The lethal and sublethal effects of four insecticides with different modes of action—pyriproxyfen, teflubenzuron, acetamiprid, and cypermethrin—were evaluated on pupae and adults of *Eriopis connexa*, an effective predator in horticultural crops. Pupal survival was reduced by pyriproxyfen (26 %) and cypermethrin (41 %). Malformations in adults emerged from treated pupae were observed after acetamiprid (82.7 and 100 % for 100 and 200 mg a.i./l, respectively), pyriproxyfen (48.6 %), and cypermethrin (13.3 %) treatments. A longer mean oviposition time was also observed in adults emerged from pupae treated with cypermethrin. Moreover, the latter insecticide as well as teflubenzuron did not reduce reproductive parameters, whereas females emerged from pyriproxyfen-treated pupae were not able to lay eggs even when females showed large abdomens. Upon exposure of adults, survival was reduced to approximately

90 % by acetamiprid, but no reduction occurred with pyriproxyfen, teflubenzuron, or cypermethrin though the fecundity at fifth oviposition time of the female survivors was reduced. Pyriproxyfen decreased the hatching at all the oviposition times tested, whereas fertility was reduced in the fourth and fifth ovipositions by teflubenzuron and in the first and third ovipositions by cypermethrin. In conclusion, all four insecticides tested exhibited lethal or sublethal effects, or both, on *E. connexa*. The neurotoxic insecticides were more harmful than the insect-growth regulators, and pupae were more susceptible than adults. The toxicity of insecticides on the conservation of predators in agroecosystems of the Neotropical Region is discussed.

Keywords *Eriopis connexa* · Insecticides selectivity · Lethal effects · Sublethal effects

Introduction

Pesticides have been used in agriculture for the last several decades in order to reduce crop damage by pests although the continuous employment of these chemicals has caused environmental contamination as well as side effects to nontarget organisms. The “Green Revolution” was a period when the productivity of global agriculture increased drastically because of new advances as chemical fertilizers and pesticides, high-yield crops, and multi-cropping system (Sánchez-Bayo 2011). For the last 25 years, however, worldwide trends have been pointing to the search for highly selective pesticides with a low impact on the environment and on human health.

Biological control is an alternative strategy to control pests rather than the use of chemicals. Biological control involves the use of natural enemies (i.e., predators, parasitoids, and entomopathogens) to reduce pest population density below the economic-injury level (Jacas and Urbaneja 2009). It

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represents an environmentally sustainable and economically profitable strategy (Cock et al. 2010). Although the use of pesticides for pest control in the developing countries of South America remains as the principal pest-management strategy (Wyckhuys et al. 2013), biological control through the conservation of beneficial arthropods would constitute an especially useful and relevant tool for maximizing ecosystem services and reducing pesticide use.

Coccinellidae predators have a relevant role in controlling pests, mainly on sap-sucking ones (Weber and Lundgren 2009; Obrycki et al. 2009; Biddinger et al. 2009; Michaud 2012). Within this family, the generalist predator *Eriopis connexa* (Genmar) is being considered as a potential biocontrol agent for several horticultural crops in the Neotropical Region (Almeida-Sarmento et al. 2007; Duarte Gómez and Zenner de Polanía 2009). In Argentina, this species is commonly found associated with key horticultural pests such as aphids and whiteflies (Fogel 2012). The conservation of *E. connexa* in these agroecosystems is, however, compromised by the indiscriminate application of pesticides, mainly broad-spectrum ones. Therefore, the introduction and application of new reduced risk chemicals are crucial. Indeed, within the pest-control strategies of integrated pest management (IPM), biological and chemical controls by selective pesticides are both worldwide promoted nowadays (Desneux et al. 2007; Stark et al. 2007; Yao et al. 2015).

Pyriproxyfen, teflubenzuron, and acetamiprid insecticides are commonly used on Argentine horticultural crops for controlling phytophagous pests and considered as selective insecticides due to having a lower environmental impact than conventional insecticides (EPA 2013). Although their effectiveness on several pests has been widely documented (Ishaaya et al. 1994; Ishaaya et al. 2001; Bacci et al. 2007), few studies are yet available indicating their potential toxicity to nontarget organisms, such as coccinellids.

Pyriproxyfen is a potent juvenile-hormone analog mimicking the authentic hormone secreted by the *Corpus allatum* in insects that causes strong suppression of embryogenesis, metamorphosis, and adult formation (Ghanim and Ishaaya 2010). Teflubenzuron, belonging to the group of benzoylureas, has insecticidal action on larvae through an inhibition of chitin synthesis that causes cuticle rupture and consequent death (Ware and Whitacre 2004). Acetamiprid is a neonicotinoid that antagonizes the central nervous systems of insects through a specific interaction with nicotinic acetylcholine receptors to produce excitation, paralysis, and death (Tomizawa and Casida 2005).

Pyriproxyfen, teflubenzuron, and acetamiprid have been considered harmless to several natural enemies of agricultural pests and, thus, are compatible with IPM programs (Ishaaya et al. 2007; Moscardini et al. 2013). A worldwide controversy exists, however, as to the selectivity of neonicotinoids, such as acetamiprid, because of the high toxicity documented for

several pollinators along with certain other beneficial insects (Grafton-Cardwell and Gu 2003; Papachristos and Milonas 2008; Blacquière et al. 2012; He et al. 2012; Fogel et al. 2013; Malagnoux et al. 2015). Moreover, neonicotinoid insecticides are frequently detected in soil and water and are also found in air, as dust particles during sowing of crops and aerosols during spraying (Chagnon et al. 2015).

Other conventional insecticides such as pyrethroids have been found to be harmful to several vertebrates such as amphibians and fish (Singh and Singh 2008; Agostini et al. 2010) along with arthropods (Desneux et al. 2004a; Schneider et al. 2006; Rimoldi et al. 2008, 2012; Benamú et al. 2013), but those products nevertheless still remain on the market. Cypermethrin, for example, has been widely used in agroecosystems in Argentina under open field and greenhouse conditions for several years, and it has not yet been replaced by environmentally friendly compounds mainly because of the high cost of biorational pesticides (Cappello and Fortunato 2008). This insecticide acts at the level of the central nervous system on axonic Na^+ channels, producing an excessive input of the ion that leads to a continuous excitation of the axonic membrane, causing repetitive discharges and eventual paralysis (Stenersen 2004).

Within this context, the aim of the present study was to evaluate, under laboratory conditions, the toxicity of pyriproxyfen, teflubenzuron, acetamiprid, and cypermethrin on the pupal and adult stages of the predator *E. connexa* according to the criteria of modern ecotoxicology, where the lethal and sublethal effects are considered equally relevant (Desneux et al. 2007; Stark et al. 2007).

Materials and methods

The insect colonies and bioassays were carried out in a growth chamber with controlled environmental conditions: temperature, 25 ± 2 °C; relative humidity, 70 ± 5 %; and photoperiod, 16:8-h (light/dark).

Insects

Eriopis connexa adults used to establish a colony in the laboratory were obtained from surveys carried out on horticultural crops within the environs of La Plata, Argentina ($34^\circ 57' 17''$ S, $57^\circ 53' 26''$ W). The bird-cherry aphid *Rhopalosiphum padi* (Linnaeus, 1758; Hemiptera: Aphididae) was used as prey, and a colony was initiated from clones obtained from the School of Agricultural and Forestry Sciences (National University of La Plata, Argentina) with the individuals being reared on pesticide-free wheat seedlings (*Triticum aestivum* L.; cultivar ACA 901). *E. connexa* adults used for testing were fed ad libitum with prey and an artificial diet based on a beef-liver nutritional supplement (Martos and Niemeyer 1990).

Insecticides

The formulated insecticides tested were Epingle® (10 % [w/v] pyriproxyfen; Summit-Agro, Buenos Aires, Argentina), Nomolt® (15 % [w/v] teflubenzuron; BASF, Buenos Aires, Argentina), Mospilan® (acetamiprid 20 % [w/w]; Summit-Agro, Buenos Aires, Argentina), and Glextrin 25® (25 % [w/v] cypermethrin; Gleba, La Plata, Argentina). For each insecticide, the following maximum recommended field concentrations (MRFCs) registered in Argentina (CASAFE 2013/2015) were assessed: pyriproxyfen, 75 mg of active ingredient (a.i.)L⁻¹; teflubenzuron, 45 mg a.i.L⁻¹; acetamiprid, 200 mg a.i.L⁻¹; and cypermethrin, 25 mg a.i.L⁻¹. For the pupal bioassay, the 50 % MRFC (100 mg a.i.L⁻¹) of acetamiprid was also evaluated, based on the results of a previous study for immature stages (eggs, second and fourth larval instars) indicating MRFC to be highly toxic to predators (Fogel et al. 2013).

Bioassays

Pupae bioassay

Three replicates of ten young pupae each (at fewer than 24 h after pupation) from the laboratory colony were used in each treatment. Each individual was exposed to 1 µl of insecticide by topical application with a hand microapplicator (Burkard®, UK). Insecticide solutions were prepared in acetone (analytical grade) as the solvent. For acetamiprid, the solutions were prepared with 20 % (v/v) acetone in distilled water (Youn et al. 2003) for improving dissolution. Controls were treated with the solvent alone.

The weight of the pupae averaged 0.43±0.07 g, so that the concentrations studied were equivalent to doses in micrograms a.i. per gram pupae of 0.17 for pyriproxyfen, 0.10 for teflubenzuron, 0.06 for cypermethrin, and 0.46 and 0.23 for acetamiprid at 100 and 50 % of the MRFC, respectively. The treated pupae were placed individually in plastic Petri dishes (9-cm diameter, 1.5-cm height) and then transferred to a growth chamber under the environmental conditions mentioned above. The following end points were evaluated daily: pupal survival (pupae with dark coloration being considered dead) and abnormal morphology in the emerged adults (i.e., malformations and abnormalities). In treatments where normal females were observed, we also measured the mean oviposition time (mean time after adult emergence and the first egg laying), cumulative fecundity (number of eggs laid), and fertility (percentage of eggs hatched) during the first 48 h after the first oviposition (according to a prior pilot bioassay).

Adult bioassay

Before exposure, five groups of 30 *E. connexa* adults (5-day-old; sex ratio 0.5:0.5) from the laboratory colony were placed in cylindrical plastic containers (18-cm diameter, 15-cm height) for 5 days to insure the effective mating of the females. Next, the adults were separated into individual ventilated plastic containers (5-cm diameter, 7-cm height) and exposed to doses of each product in drinking water at the MRFCs indicated above. The solutions were changed daily for 3 days (Viñuela et al. 2001), after which time the individuals were provided with distilled water. Each treatment consisted of three replications of 10 adults. For a total of 15 days, we assessed the survival of the adults and the reproductive capacity of the females daily. From this, the adult cumulative survival at the end of the bioassay was determined, and the mean oviposition time and the fecundity and fertility of the females during the first five ovipositions (that normally occurred during the first 15 days from adult emergence) were recorded. The reproductive parameters were evaluated in the treatments where more than 12 females survived (the minimum number for statistical validity).

Statistical analysis

Results are presented as the means±standard error. Before data analysis, the Shapiro-Wilk test was used to assess data normality. One-way analysis of variance (ANOVA) or the repeated-measures ANOVA test was used to ascertain differences between treatments. When necessary, a log-data transformation [$y = \log_{10}(x + 1)$] or an angular transformation ($p' = \arcsine p^{1/2}$) were done (Zar 1996). After ANOVA, the separation of the means was assessed by a multiple-range test (LSD; $P < 0.05$). Survival analysis was used to determine the mean oviposition time with each treatment. The *survival functions* were estimated by the Kaplan-Meier method along with the Log-rank test for treatment comparisons, through the use of the Bonferroni correction for paired comparisons between treatments. The XLStat program (Addinsoft XLstat for Excel, Paris, France. 2009. <http://xlstat.softonic.com>) was used in the analyses.

Results

Pupae bioassay

The conventional insecticide cypermethrin produced the highest toxicity among the pesticides tested, reducing the survival of the pupae by 41 %. The biorational insecticide pyriproxyfen also significantly diminished pupal survival by 26 % (Table 1). Neither of the other insecticides caused a significant degree of pupal mortality.

Table 1 Side effects of pyriproxyfen, teflubenzuron, cypermethrin, and acetamiprid on the survival and teratology of *Eriopsis connexa* from the topical bioassay on the pupal stage

Treatments	Concentration (mg a.i. L ⁻¹)	Survival pupae (%)	Adults malformed ^b
Control	0	100±0.0 a	0±0.0 a
Pyriproxyfen	75 ^a	74.0±7.4 bc	48.6±8.4 b
Teflubenzuron	45 ^a	81.5±7.4 ab	0±0.0 a
Cypermethrin	25 ^a	59.2±3.7 c	13.3±6.6 a
Acetamiprid	200 ^a	85.2±9.7 ab	100±0.0 c
Acetamiprid	100	95.8±4.1 a	82.7±10.5 c

The data correspond to means (±SE). Different letters within columns denotes significant differences between treatments. One-way ANOVA ($p \leq 0.05$). Surviving pupae: $F = 5.59$; $df = 5, 17$; $P \leq 0.007$. Malformed adults: $F = 43.3$, $df = 5, 17$, $P \leq 0.0001$

^a Maximum recommended field concentration

^b Percentage of total adults malformed

Acetamiprid, pyriproxyfen, and cypermethrin caused malformations in adults emerged from treated pupae. In detail, 100 and 82 % of the adults emerging from pupae treated with acetamiprid at the MRFC and 50 % of the MRFC, respectively, exhibited malformations, whereas 49 and 13 % of adults emerging from pupae treated by pyriproxyfen and cypermethrin showed malformations, respectively. In contrast, no malformed adults emerged after pupae exposure to the MRFC of teflubenzuron (Table 1).

Acetamiprid (at 100 and 50 % of the MRFC) and pyriproxyfen (at the MRFC) caused similar severe malformations on individuals after pupal exposure. In acetamiprid treatments, diverse types of malformations involving intermediate stages of development were observed—i.e., organisms with their anterior part consisting of an adult head, but having the thorax and abdomen of a pupa (Fig. 1b). Other individuals manifested partially developed elytra that adhered to the rest of pupal cuticle or individuals that exhibited smaller elytra that did not cover their thorax and abdomen (Fig. 1c, d). Pyriproxyfen also disrupted normal metamorphosis (from pupa to adult), observing intermediate stages of development and some adults emerged malformed—i.e., having divided elytra with an inability to discard the remains of the puparium: Fig. 1e, f).

Effects on the reproductive parameters of adults could be assessed only when enough normal adults (without malformation) were recovered. Although the assessment in adults emerging after pupal acetamiprid treatment was therefore impossible, the evaluation could be performed for the other insecticides assayed (Table 1). Accordingly, the organisms exposed to cypermethrin had higher mean oviposition times than the controls in the form of a delay in the time to the first oviposition. Nevertheless, no effect was observed on the cumulative fecundity and fertility at 48 h (Table 2). Teflubenzuron,

however, affected neither the mean oviposition times nor the cumulative fecundity and fertility (Table 2). Although 50 % of the pupae treated with pyriproxyfen developed into anatomically normal adults, the females failed to lay eggs in spite of the development of their ovaries, as assessed on the basis of their notably large abdomens (Table 2).

Adults bioassay

Acetamiprid significantly reduced the cumulative survival of the *E. connexa* adults (Fig. 2) with the shortest time to causing effects (all organisms dead 24 h after exposure), whereas pyriproxyfen, teflubenzuron, and cypermethrin caused no significant change in survival, thus enabling an evaluation of the subsequent effects on reproductive parameters. None of the insecticides tested affected the mean oviposition time ($\log \text{rank} = 3.174$, $df = 3$, $P = 0.366$), while the effects on the fecundity and fertility were dissimilar. The adults exposed to pyriproxyfen and cypermethrin laid more eggs during the first oviposition than the controls. While in the fifth oviposition, the fecundity declined significantly. Teflubenzuron reduced the fecundity in only the fifth oviposition, whereas the fecundity in the other ovipositions was similar to that of the controls (Table 3). A reduction in the egg hatches (fertility) was detected after treatment with pyriproxyfen in all of the ovipositions, while lower percentages of hatching were also observed in the first and third oviposition of the adults exposed to cypermethrin. Teflubenzuron, however, produced significant effects on fertility in only the third oviposition (Table 3).

Discussion

Agro-ecosystems are continuously exposed to pesticides through pest management by farmers, which is a practice that contributes to a loss in the biodiversity within these environments (Gibbs et al. 2009), reducing the population density of nontarget organisms, as species that feed on phytophagous pests.

The biological control of pests by natural enemies has a low environmental impact, and it has been promoted in conjunction with their use of selective pesticides in IPM programs to enhance the biodiversity of agricultural ecosystems. In the past few decades, however, many studies have reported either negative or lethal and/or sublethal effects of pesticides on beneficial nontarget organisms including natural enemies of agricultural pests (Schneider et al. 2003, 2004, 2006; Desneux et al. 2007; Ronco et al. 2008; Biondi et al. 2012; Benamú et al. 2013). Nevertheless, the side effects of pesticides on Coccinellidae predators have not been deeply documented yet.

The present study contributes with novel ecotoxicological data of a neonicotinoid, acetamiprid; a pyrethroid,

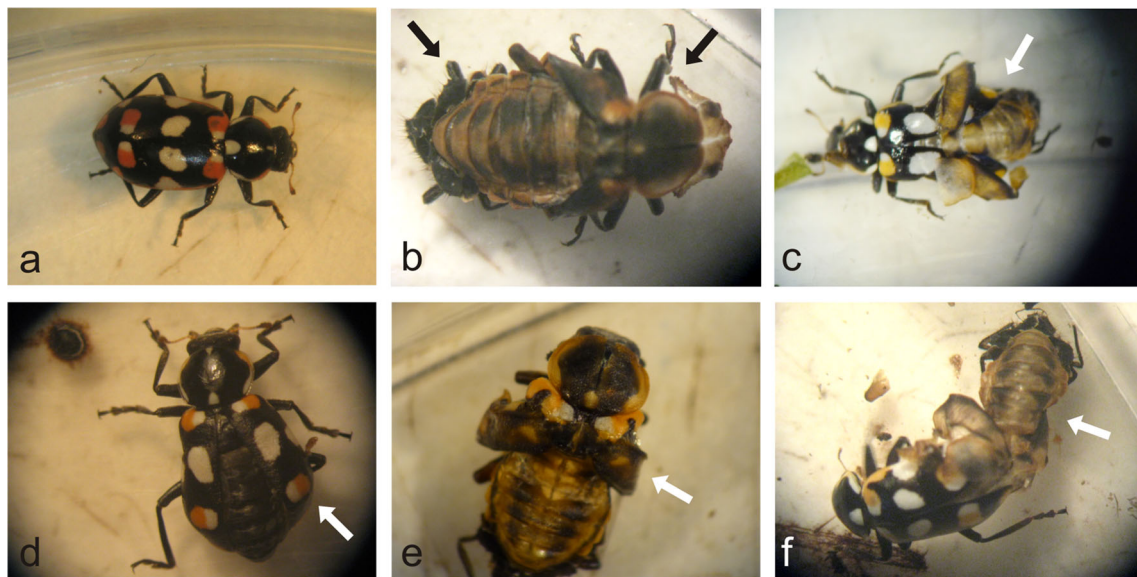


Fig. 1 Malformations in *Eriopis connexa* after topical exposure of pupae. **a** Control adult, **b, c** acetamiprid (200 mg L⁻¹ a.i.), **d** acetamiprid (100 mg L⁻¹ a.i.), **e, f** pyriproxyfen (75 mg L⁻¹ a.i.). Arrows signalize the main malformations observed

cypermethrin, and two IGR insecticides, pyriproxyfen, and teflubenzuron, on the coccinellid *E. connexa*. All insecticides affected several biological parameters of predator but to our knowledge, this is the first report on neurotoxic insecticides acetamiprid and cypermethrin to cause malformations and impair metamorphic processes in pupae of *E. connexa*.

Effects of insecticides on pupae

In the present study, pyriproxyfen caused adverse effects on the treated pupae of *E. connexa* that affected pupal survival. These results agree with those from other reports on various natural enemies of pests (Chen and Liu 2002; Schneider et al.

2004; Planes et al. 2013). Furthermore, we also recorded malformations and/or other abnormalities in the emerged adults. Malformed organisms associated with pyriproxyfen exposure have been reported in various beneficial arthropods—e.g., *Rodolia cardinalis* Mulsant (Mendel et al. 1994), *Podisus maculiventris* Say (Mestdagh et al. 1996), and *Encarsia formosa* Gahan (Darvas and Polgar 1998). As for reproductive parameters, we observed that females of *E. connexa*, though exhibiting large abdomens, were unable to lay eggs during the assessment period so that the mean oviposition time with those adults could not be measured. Pyriproxyfen is known to mimic the action of the juvenile hormone during the immature stages of insects to induce disruptions during metamorphosis, suppress embryogenesis, and stunt and modify adult formation (Ishaaya et al. 1994). We therefore expected to observe malformations in the pupal stage and ostensibly normal female adults not capable of laying eggs (fecundity inhibition).

Although the neonicotinoid central nervous system antagonist acetamiprid did not cause mortality after pupal exposure, malformations occurred during metamorphosis. On the basis of our results, the observed malformations during metamorphosis could be explained upon consideration that the central nervous system regulates the synthesis of neurohormones that, in turn, control different enzymatic processes, whose actions finally determine the degree of growth and the extent and nature of developmental hormone production. Several authors have reported sublethal effects of neonicotinoids on the natural enemies of pests—such as an immobility of predator *Deraeocoris brevis* (Uhler) (Kim et al. 2006), neurotoxic symptoms such as tremors, paralysis, and loss of coordination on the predator coccinellid *Hippodamia undecimnotata*

Table 2 Effect of pyriproxyfen, teflubenzuron, and cypermethrin on the reproductive parameters of *Eriopis connexa* adult survivors from the topical bioassay on the pupal stage

	Concentration (mg a.i. L ⁻¹)	Mean oviposition time (days)	Cumulative fecundity ^a	Cumulative fertility ^b
Control	0	6.6 ± 0.1 a	36.7 ± 3.8 a	85.1 ± 4.6 a
Pyriproxyfen	75	–	0 ± 0.0 b	–
Teflubenzuron	45	7.1 ± 0.3 a	28.4 ± 5.9 a	70.3 ± 9.1 a
Cypermethrin	25	8.4 ± 0.4 b	35.5 ± 6.7 a	75.5 ± 11.9 a

The data correspond to means (±SE). Different letters within columns denote significant differences between treatments; $P \leq 0.05$. Mean oviposition time: $df=2$, $P \leq 0.04$, long rank = 6.423. Cumulative fecundity: $F = 16.77$; $df=3, 26$; $P \leq 0.0001$. Cumulative fertility: $F = 1.25$; $df=2, 19$; $P = 0.311$. One-way ANOVA ($P = 0.05$)

^a Fecundity: Mean number of eggs laid/female

^b Fertility: Percentage of eggs hatched/female

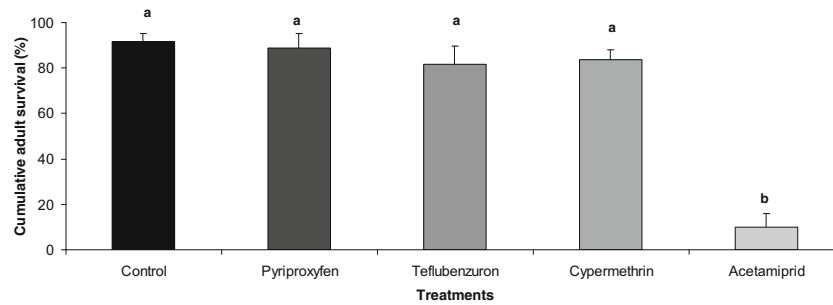


Fig. 2 Effect of pyriproxyfen, teflubenzuron, cypermethrin, and acetamiprid on the cumulative survival (15 days) of *Eriopis connexa* after adult treatments by ingestion (through the drinking water). Data

correspond to means (\pm SE). Treatments with different letters are significantly different (one-way ANOVA $P < 0.05$) at $F = 38.3$, $df = 4, 15$, $P \leq 0.0001$

(Schneider) (Papachristos and Milonas 2008), a shortening of the developmental period on ladybird beetle, *Coccinella septempunctata* L. (Yu et al. 2014), and a decrease in the rate of food consumption of predator *Serangium japonicum* Chapin (He et al. 2012). However, malformations as sublethal effects of this insecticide have been not reported yet, and the present report constitutes the first documentation of malformations of a neonicotinoid insecticide on *E. connexa* that indicate either a direct or an indirect effect on metamorphosis, though the mechanisms involved have yet to be elucidated.

The pyrethroid cypermethrin produced significant effects on the survival of *E. connexa* pupae. Our results agree with studies from several investigators reporting reduced larval stages and pupal survival in other natural enemies after exposure to this insecticide (Lorca Gonzalez 2005; Rimoldi et al. 2008; Garzon et al. 2015). With cypermethrin-treated pupae,

although the fecundity and fertility upon reaching the adult stage were not affected, the mean oviposition time was delayed. Similar results had been obtained with cypermethrin on the predators *Chrysoperla externa* Hagen and *P. maculiventris* (Mohaghegh et al. 2000; Rimoldi et al. 2012).

No effects were observed on the survival of the pupae, the morphology of the emerged adults, or the reproductive parameters of *E. connexa* after pupal exposure to teflubenzuron. The lack of toxicity of this insecticide and other chitin inhibitors has been extensively documented for other arthropod predators: *Chrysoperla carnea* Stephens (Medina et al. 2003), *Harmonia axyridis* Pallas (James 2004), and the parasitoids *Aphytis melinus* DeBach (Rill et al. 2008) and *Encyrtus infelix* Embleton (Mendel et al. 1994). Schneider et al. (2003), however, reported a reduction in reproductive parameters of another parasitoid species *Hyposoter didymator* Thunberg upon pupal exposure to the chitin-synthesis inhibitor diflubenzuron.

Table 3 Effect of pyriproxyfen, teflubenzuron, and cypermethrin on reproductive parameters of *Eriopis connexa* from bioassay on the adult stage

	Concentration (mg a.i. L ⁻¹)	First oviposition	Second oviposition	Third oviposition	Fourth oviposition	Fifth oviposition
Fecundity ^a						
Control	0	17.2 \pm 1.4a	16.7 \pm 1.4a	15.0 \pm 1.6a	17.0 \pm 2.6a	25.1 \pm 3.7a
Pyriproxyfen	75	20.6 \pm 3.6b	17.6 \pm 4.1a	17.8 \pm 3.1ab	13.0 \pm 4.1a	12.0 \pm 4.0b
	45	18.6 \pm 4.4a	19.0 \pm 3.0a	18.6 \pm 1.3ab	16.9 \pm 3.1a	12.1 \pm 4.7b
Teflubenzuron						
Cypermethrin	25	22.3 \pm 2.3b	14.9 \pm 2.6a	12.0 \pm 1.7bc	16.8 \pm 2.8a	10.6 \pm 2.5b
Fertility ^b						
Control	0	61.8 \pm 7.3a	70.2 \pm 5.1a	80.9 \pm 4.9a	68.2 \pm 6.7a	60.9 \pm 7.4a
Pyriproxyfen	75	42.3 \pm 8.9c	41.8 \pm 8.0b	50.0 \pm 10c	48.7 \pm 9.1b	48.9 \pm 9.0b
	45	62.7 \pm 10.5a	63.0 \pm 8.7a	62.6 \pm 7.2b	54.7 \pm 12.7b	63.5 \pm 12.7a
Teflubenzuron						
Cypermethrin	25	72.2 \pm 7.1b	63.1 \pm 9.1a	66.9 \pm 5.8b	66.1 \pm 5.8a	62.9 \pm 7.4a

The data correspond to means (\pm SE). Treatments with different letters within columns are significantly different. Repeated measures ANOVA ($P \leq 0.05$). Fecundity: $F = 1.502$; $df = 19, 235$; $P \leq 0.086$. Fertility: $F = 1.724$, $df = 19, 215$, $P \leq 0.034$

^a Fecundity: Mean number of eggs laid/female

^b Fertility: percentage of eggs hatched/female

Effects of insecticides on adults

Acetamiprid caused a significant increase in mortality of *E. connexa* adults after ingestion via drinking water. Several studies with acetamiprid and other neonicotinoids had observed similar effects on several Coccinellidae species such as *H. axyridis* (Youn et al. 2003; Moser and Obrycki 2009), *R. cardinalis* (Grafton-Cardwell and Gu 2003), *Serangium japonicum* Chapin (He et al. 2012), and *H. undecimnotata* (Papachristos and Milonas 2008). The present results corroborate those of prior studies with eggs and larvae of this predator and confirmed the effects of this pesticide on all development stages of *E. connexa* (Fogel et al. 2013).

In the present study, pyriproxyfen and teflubenzuron did not affect the survival of *E. connexa* adults; however, the negative impact on reproductive parameters, though the effects by teflubenzuron were significantly lower. Liu and Stansly (2004) had observed deleterious effects on the fertility of *Delphastus catalinae* Horn in adults fed with pyriproxyfen-treated whitefly eggs. Nevertheless, this insecticide was harmless to adults of *R. cardinalis* after exposure via ingestion, though this insecticide reduced the number of progeny (Grafton-Cardwell and Gu 2003).

After oral ingestion, cypermethrin did not affect the survival of *E. connexa* adults. By contrast, several studies had reported toxicity of pyrethroids in adult predators and parasitoids (Grafton-Cardwell and Gu 2003; Desneux et al. 2004b; Rimoldi et al. 2012). This discrepancy between our and other studies could be attributed to the well-known repellent effect of pyrethroids on arthropods; accordingly, the absence of toxicity, in fact, could have resulted from an actual low exposure to the active ingredient (Desneux et al. 2005; Benamú et al. 2013). On the other hand, this insecticide drastically reduced fecundity and fertility of *E. connexa*, agreeing with studies reported by Rimoldi et al. (2012), who observed a decrease in the fertility of the adult *C. externa*.

Insecticides and IPM programs

The pest control strategies of the IPM paradigm include chemical intervention by selective pesticides and alleges compatibility between the pest-regulatory strategies employed (i.e., biological and chemical control). The lethal and sublethal effects observed in this study on the predator *E. connexa* become highly relevant to this criterion of compatibility and must be taken into account when considering strategies for conserving this species as a potential biological-control agent in horticultural crops of the Neotropical Region.

In conclusion, taking into account the results obtained on both development stages of the test species and considering the framework of the IPM paradigm, acetamiprid caused both lethal and sublethal effects, whereas pyriproxyfen and cypermethrin mainly induced sublethal effects.

Teflubenzuron was the less toxic tested insecticide toward *E. connexa*. The results reported here highlight the relevance of these insecticide effects not only on the directly exposed organisms but also on their progeny, causing a reduction in the size of it that could impact on the predator population dynamics and conservation of it in agroecosystems.

Regarding the high toxicity and sublethal effects of the neonicotinoid acetamiprid on *E. connexa*, it is recommended that this insecticide should not be included in IPM programs involving this predator as biocontrol agent.

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