



Lethal and sublethal effects of seven insecticides on three beneficial insects in laboratory assays and field trials



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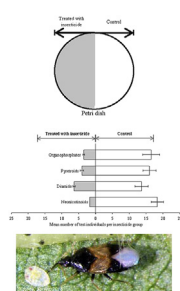
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HIGHLIGHTS

- Safety of seven pesticides to the three beneficial insects was evaluated.
- The chlorantraniliprole showed low lethal and sublethal effects to test-species.
- Deltamethrin induced hormesis in *Cycloneda sanguinea* and *Orius insidiosus*.
- The organophosphates and pyrethroids on predators IPM be evaluated with caution.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 4 February 2016

Received in revised form

26 April 2016

Accepted 26 April 2016

Handling Editor: David Volz

Keywords:

Agroecosystems
Ecotoxicology
Natural enemies
Pesticides
Toxicity

ABSTRACT

Lethal and sublethal effects of insecticides on target and non-target arthropods are a concern of pest management programs. *Cycloneda sanguinea*, *Orius insidiosus* and *Chauliognathus flavipes* are important biological control agents for aphids, whitefly, lepidopterus eggs, thrips and mites. All three test species were subjected to a toxicity study using the insecticides acephate, bifenthrin, chlorantraniliprole, chlorpyrifos, deltamethrin, imidacloprid, and thiamethoxam. Experiments were done in the lab and field. In the laboratory we evaluated the mortality and sublethal effects of the concentration that killed 20% of the population (LC₂₀) on feeding, repellence and reproduction of the species tested. The lethal effects of these insecticides at the recommended doses was evaluated in the field. Concentration-response bioassays indicated chlorantraniliprole had the lowest toxicity, while chlorpyrifos and acephate were the most toxic. Test species exposed to filter paper surfaces treated with pyrethroids, neonicotinoids and organophosphates were repelled. On the other hand, test species were not repelled from surfaces treated with chlorantraniliprole. Chlorantraniliprole therefore seemed to be the least dangerous insecticide for these three beneficial arthropod test species.

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1. Introduction

A key element of pest management programs in agroecosystems is to build an understanding of the impacts on non-target and

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beneficial insects (Desneux et al., 2007). The use of insecticides against insect-pests still prevails as one of the main pest management tools in most agricultural settings, in addition to having potential consequences for arthropod pest resurgence (Roubos et al., 2014).

Insecticides may block some physiological or biochemical processes, impacting survival, growth, development, reproduction and behavior of natural enemies of insect pests (Desneux et al., 2007; Castro et al., 2012). Even at non-lethal levels, insecticides can still influence behavior, although there have been few detailed studies concerning the potential effects of sublethal insecticide doses on the behavior of beneficial arthropods. In general, sublethal insecticides levels affect reproduction, orientation, feeding, oviposition and learning. In many cases insecticides act as repellents associated with food searching behavior. Repellency may result from contact with the host or prey treated with insecticides (Saran et al., 2014). Moreover, there is the possibility that a phenomenon known as hormesis will occur in populations of natural enemies (Guedes and Cutler, 2014). Hormesis is defined as the stimulation of organism performances that occur at low levels of exposure to agents that are normally toxic at high levels of exposure (Calabrese and Baldwin, 2001). This phenomenon has been reported for many animal-toxicant models and has often been suggested as the main mechanism for pest population resurgence (Cordeiro et al., 2013; Qu et al., 2015).

Pyrethroids and organophosphates were introduced in the mid 1980s, followed by the neonicotinoids in 1990 (Grube et al., 2011). Compared to these insecticides, the anthranilic diamides (e.g. chlorantraniliprole) were commercialized in 2006 (Lahm et al., 2009). The oldest insecticides, deltamethrin and bifenthrin (pyrethroids); imidacloprid and thiamethoxam (neonicotinoids) and chlorpyrifos and acephate (organophosphates), are reported to cause lethal and sublethal effects on natural enemies (Desneux et al., 2007; Rajak et al., 2014), but in the majority of cases the diamides showed lesser effects on this group (Brugger et al., 2000; Moscardini et al., 2015). Pyrethroids act as sodium channel modulators, organophosphates inhibit the action of the acetylcholinesterase enzyme, neonicotinoids bind and act as agonists on acetylcholine receptors (postsynaptic nicotinic acetylcholine receptor), causing paralysis that leads to death, often within a few hours and the diamides modulate the calcium channels connecting the ryanodine receptors (IRAC, 2015). In ambient conditions, the groups cited may be degraded by biotic (Zuo et al., 2015) and abiotic action (Sharma et al., 2014).

Families such as Coccinellidae and Anthicoridae have received attention because of their importance as natural enemies to some major insect pest species. Cantharidae, on the other hand, has not been well studied. *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) and *Orius insidiosus* (Say) (Hemiptera: Anthicoridae) are important biological control agents for aphids, whitefly, lepidopteran eggs, thrips and mites (Oliveira et al., 2005; Lucas, 2012; Yamada et al., 2016), but little is known about *Chauliognathus flavipes* Fabricius (Coleoptera: Cantharidae). Boiça Junior et al. (2004) observed that in greenhouse conditions, *C. sanguinea* adults reduced the population of *Aphis gossypii* Glöver (Hemiptera: Aphididae) by 93.5% in 2 d. In the United States, *O. insidiosus* is a significant factor in the predictable seasonal declines in *Frankliniella* spp., following population peaks and is able to suppress *F.* spp. populations in *Capsicum annum* L. (Reitz et al., 2003). There is little information on the predation capacity of *C. flavipes* and its use in programs of biological control, but the Cantharidae family is an important predator of aphids (Berthiaume et al., 2001).

Considering the importance of the insecticides and the above predators, the objective was to (1) evaluate the effect of the insecticides chlorpyrifos, chlorantraniliprole, deltamethrin, acephate,

imidacloprid, bifenthrin and thiamethoxam on mortality in laboratory and field conditions, and (2) to evaluate the effect of the sublethal doses of these insecticides on the feeding, repellence and reproductive behavior of *O. insidiosus*, *C. sanguinea* and *C. flavipes*.

2. Materials and methods

2.1. Insects

Adults of *O. insidiosus*, *C. sanguinea* and *C. flavipes* were collected from corn (*Zea mays* L.), soy (*Glycine max* L.) and tomato (*Solanum lycopersicum* L.) plantations in experimental fields at the Universidade Federal de Viçosa, Minas Gerais State, Brazil (20°45'25"S, 42°52'55"W). The capture method used soda bottles with rectangular openings (15 × 20 cm) closed with a thin organza tissue. On the inside of the bottle a paper towel was added to facilitate insect movement. The test species were captured with a simple sucker hose, blown to the inside of the bottle and transported to the laboratory to initiate breeding.

O. insidiosus adults were raised from Mendes and Bueno (2001), with adults collected from corn plants (*Z. mays*) and separated into pairs. After separation, individuals were placed on acrylic Petri dishes (15 × 2.1 cm) sealed with polyethylene film to prevent escape. Nymphs of thrips (*F.* spp.) were used as a food source and *Bidens pilosa* L. (Asteraceae) inflorescences were used as oviposition sites. The inflorescence was observed daily with a stereoscopic microscope to verify the presence of test species eggs. The test species were kept in climatic chambers at 25.00 ± 1.00 °C, 70.00 ± 10.00% relative humidity and photoperiod of 12:12 (light:dark). The inflorescences containing *O. insidiosus* eggs were removed and collected on other Petri dishes (15 × 2.1 cm) sealed with polyethylene film. To avoid egg and nymph mortality by desiccation, a cotton ball moistened with distilled water was placed inside the dish. To avoid water condensation in the insect breeding environment, only five inflorescences of *B. pilosa* were added per container. The containers were observed three times per week to add food and to moisten the cotton. The inflorescences of *B. pilosa* used as substrate for oviposition of *O. insidiosus* females were kept in the container to provide shelter to the nymphs. When the nymphs transformed into adults they were removed to perform the bioassays.

C. sanguinea adults were raised according to Oliveira et al. (2005) and *C. flavipes* adults were collected from soy plants. Adults collected from the field were individually transferred to plastic pots (250 mL), which had 1 cm² circular perforations to allow gas exchange with the external environment. A paper towel was added to the inside of the pots so the adults could oviposit. The adults were removed after oviposition and the eggs were placed on glass Petri dishes (15 × 2.0 cm) with a small moist cotton ball dampened with sanitary water added to prevent the eggs from drying out. After hatching, larvae were individually transferred to 500 mL plastic pots, with about 15 aphids/pot/larva/day. When the larvae pupated they were transferred to a BOD (Biochemical Oxygen Demand) germinating chamber at a constant temperature of 25.00 ± 0.10 °C and relative humidity of 75.04 ± 0.40% to hatch the adults that were then used in the bioassays.

2.2. Insecticides

Commercial formulations of seven neurotoxic insecticides available for use on soy, corn and tomato fields in Brazil were used. These were the organophosphates chlorpyrifos (Lorsban 480 emulsifiable concentrate; i.e., containing 480 g a.i. L⁻¹, Dow Agrosiences industrial Ltda, São Paulo, SP, Brazil) and acephate (Orthene 750 water-dispersible granules, i.e. containing 750 g a.i.

L⁻¹, Arysta Lifescience Corporation, São Paulo, SP, Brazil); the neonicotinoids thiamethoxam (Actara 250 water-dispersible granules, i.e. containing 250 g a.i. L⁻¹, Syngenta Ltda, Barueri, SP, Brazil) and imidacloprid (Imidacloprid Nortox 480 suspendable concentrate, i.e. containing 480 g a.i. L⁻¹, Nortox S.A., Arapongas, PR, Brazil); the pyrethroids deltamethrin (Keshet 25 emulsifiable concentrate, i.e. containing 25 g a.i. L⁻¹, Bayer Crop Science Ltda, São Paulo, SP, Brazil) and bifenthrin (Talstar 100 emulsifiable concentrate, i.e. containing 100 g a.i. L⁻¹, FMC Brazil, Campinas, Brazil) and a new anthranilic diamide insecticide, chlorantraniliprole (Premio 200 emulsifiable concentrate, i.e. containing 200 g a.i. L⁻¹, DuPont Brazil, São Paulo, Brazil).

2.3. Filter paper lethal toxicity tests

The insecticides were diluted with distilled water forming a stock solution (made fresh for each experiment), which was used to provide serial dilutions. Different concentrations of each insecticide were used in addition to the control, which used water only were considered dead if unable to move when to correct for natural mortality (Table 1). Filter paper disks (9.3 cm diameter) were immersed for 10 s in insecticide solution and shade dried for 30 min, then applied to the inner surface of Petri dishes (9 × 2 cm), adapted from He et al. (2012). The Petri dishes were subsequently placed with inner walls coated with Teflon PTFE (Du Pont, Wilmington, DE) to prevent test species from escaping. Ten adults of each test species were placed on each (open) Petri dish, which was placed in a rearing chamber at 25.00 ± 0.50 °C, 75.00 ± 5.00% relative humidity and photoperiod of 12:12 (light:dark). Insect mortality was assessed after 36 h of exposure and the insects prodded with a fine paintbrush. Five replicates were used for each concentration and insecticide, with the bioassays carried out following a completely randomized design. The bioassays for each

insecticide were done simultaneously under the same conditions as detailed above.

2.4. Sublethal effects on test species

The sublethal effects of the insecticides were tested on three types of behavior (feeding after contact with insecticides, repellency and reproduction) of the three test species using bioassays.

2.4.1. Feeding after contact with insecticides

A solution containing the concentration equivalent to LC₂₀ of each insecticide (Table 3) was applied topically to each test species using a micro syringe (Hamilton 10 µL), in volumes of 1, 5 and 10 µL on the species *O. insidiosus*, *C. sanguinea* and *C. flavipes*. These volumes were defined according to the body size of the test species. The control treatment was done with distilled water and the anionic surfactant polyoxyethylene alkyl phenol ether (0.15 mL L⁻¹) (Haiten 200, Arysta, Brazil). The insects were released separately into Petri dishes (10 × 2 cm), left for 36 h and the survivors were removed to perform the second bioassay.

The second bioassay was carried out with a completely randomized experimental design in a double factorial scheme (8 × 3), with seven insecticides and the control; three test species, with ten repetitions. The experiments were conducted in Petri dishes (10 × 2 cm) lined with a thin layer of solidified agar to protect the soy leaf discs (10 cm diameter) from desiccation. A single soy leaf disc was centered upside down on the agar. Prey nymphs (*F. spp.*, Thysanoptera: Thripidae and *A. gossypii* Glöver, Hemiptera: Aphididae) were collected from non-commercialized soy fields (without insecticide applications) and offered at densities of 50, 30 and 50, for the *O. insidiosus*, *C. sanguinea* and *C. flavipes* (topically treated with insecticides and control). Prey species densities for all test species were determined by a preliminary study. In order to check

Table 1

Concentrations of insecticides and number of test species *Cycloneda sanguinea*, *Orius insidiosus* and *Chauliognathus flavipes* used in bioassays.

Insecticides (mg a.i. cm ⁻²) ^a	Test species			
	<i>Cycloneda sanguinea</i>			
	Range concentration (mg a.i. cm ⁻²) ^b	Number of concentrations ^b	Number of insects ^c	Pairs of insects ^d
Chlorpyrifos (1.4 × 10 ⁴)	0.0173–0.3086	10	4020	8
Chlorantraniliprole (4.0 × 10 ⁵)	0.1573–4.7181	8	3220	10
Deltamethrin (5.0 × 10 ⁴)	0.0031–0.4718	6	2420	6
Acephate (3.7 × 10 ⁶)	0.0002–0.4718	7	2820	8
Imidacloprid (9.6 × 10 ⁵)	0.1573–1.1009	8	3220	4
Bifenthrin (3.7 × 10 ⁵)	0.0031–0.4718	10	4020	10
Thiamethoxam (2.5 × 10 ⁵)	0.0079–0.6291	12	4820	12
<i>Orius insidiosus</i>				
Chlorpyrifos	0,0016–0,3145	8	3220	10
Chlorantraniliprole	0,0157–6,2908	10	4020	20
Deltamethrin	0,0016–0,6291	10	4020	10
Acephate	0,0000–0,4718	8	3220	20
Imidacloprid	0,0002–0,3145	8	3220	6
Bifenthrin	0,0031–0,7863	10	4020	8
Thiamethoxam	0,0173–0,9436	9	3620	8
<i>Chauliognathus flavipes</i>				
Chlorpyrifos	0,0016–0,6291	10	4020	8
Chlorantraniliprole	0,0157–11,798	10	4020	8
Deltamethrin	0,0016–0,6291	8	3220	6
Acephate	0,0008–0,3145	8	3220	10
Imidacloprid	0,0001–0,2359	8	3220	12
Bifenthrin	0,0008–1,1795	8	3220	10
Thiamethoxam	0,0157–0,9436	10	4020	12
Total range	0.0000–11,798	6–12	2800–4000	6–20

^a Number of insecticides = 7 (to all bioassays) with insecticide concentrations in field experiments shown in parentheses.

^b Filter paper lethal toxicity tests (Bioassays: lethal toxicity test, repellency).

^c Sum of insects from filter paper lethal toxicity test bioassays (four repetitions) and repellency (20 repetitions).

^d Pairs of insects to reproduction bioassay.

prey nymph survival in the absence of the test species, the same number of replicates without test species was set up for each prey density. The test species were added to experimental areas 1 h after the prey nymphs were transferred. The Petri dishes were sealed with parafilm around the edges to prevent test species from escaping. The number of consumed prey was counted 5 h after the test species were released into the experimental arenas. Consumed prey were not replaced during the experiments.

2.4.2. Repellency (paired choice bioassay)

A filter paper was placed on a Petri dish (10 × 2 cm), with one half of the dish treated with a dosing solution of insecticide (LC₂₀) (following item 2.3) and the other half treated with distilled water and adjuvant anionic surfactant polyoxyethylene alkyl phenol ether (0.15 mL L⁻¹) (control). Ten adults of each test species were released in the center of the filter paper/Petri dish. Twenty replicates, each consisting of 10 insects, were used for the experiments, for a total of 200 test species per treatment. The filter paper was replaced for each trial and replicate. The number of each test species on each side of the filter paper was evaluated 15 min after the adults were released. The coffee leaf was replaced for each trial and replicate.

2.4.3. Reproduction

The fifth-instar larvae/nymphs of the test species were collected from the cages and reared individually until adulthood. The test species was exposed to the LC₂₀ of each topically-applied insecticide (Table 2) and insect survival was recorded after 60 h exposure; water-treated insects were used as control. Each virgin couple of each test species was kept in a cage (10 × 15 cm) for copulation and progeny production (Table 1). A total of 15 couples was used for each insecticide treatment (and control) and the obtained progeny was reared in glass bottles (25 × 25 cm) containing aphids as prey and paper towels under controlled conditions (25.00 ± 1.00 °C, 12:12 light:dark). The 2nd generation progeny of each initial couple was daily checked and transferred to new glass bottles until the females. The pupae were individualized in glass bottles, the emerging adults were inspected thoroughly, and their egg production was recorded (as were the unhatched eggs). Any adult female that did not produce eggs was considered to have failed to mate and the rate of successful mating (the copulation rate) was recorded. Fecundity was recorded as the average number of eggs produced by mated females and viability was calculated as (total neonates)/(total neonates plus all unhatched eggs). The number of offspring per female was reported. For the control and all insecticide treatments, 10 neonates of the new generation were reared in glass bottles (25 × 25 cm) with aphids under controlled conditions (25.00 ± 1.00 °C, 12:12 light:dark). Aphids were provided every 2 d until adult emergence. For the control and all insecticide treatments, the numbers of females and males were recorded and the sex ratio was determined.

2.5. Toxicity under field conditions

This study was conducted in 2013 and 2014 in experimental fields in the Alto Paranaíba region, Minas Gerais State, Brazil (19°12'4.90"S, 46° 7'44.09"O). The climate of the Alto Paranaíba is of tropical type (Aw according to the Köppen classification), with annual average temperatures between 8 and 28 °C per year, 60% relative humidity, average annual rainfall from 300 to 1400 mm, average altitude of 1300 m and an average wind velocity of 10.5 m s⁻¹. The study was done in an area of *S. lycopersicum* cultivar Deborah (45 d old). This crop was selected for the experiment in field conditions because it is planted in various regions of Brazil and around the world, tolerates excessive insecticide spraying and provides food and shelter for *C. sanguinea*, *O. insidiosus* and

C. flavipes (Picanço et al., 2007). The spacing between plants was 0.60 × 0.35 m (total area: 3800 m²). The area was surrounded by pasture and native vegetation. The tomato plants were kept insecticide-free for 45 d and all the cultural practices (thinning, fertilizing, staking and fungicide spraying) were performed according to (Silva and Vale, 2007).

The experiment was conducted in a randomized block design (10 × 10 m) per plot with eight treatments (seven insecticides + control) and four replications (one spray per treatment). The insecticides (treatments), rates and recommended dosages were: chlorpyrifos (1.44 g a.i. ha⁻¹, 1500 mL ha⁻¹), acephate (375 g a.i. ha⁻¹, 500 g ha⁻¹), thiamethoxam (25 g a.i. ha⁻¹, 100 g ha⁻¹), imidacloprid (96 g a.i. ha⁻¹, 200 g ha⁻¹), deltamethrin (5 g a.i. ha⁻¹, 200 mL ha⁻¹), bifenthrin (37.5 g a.i. ha⁻¹, 375 mL ha⁻¹), and chlorantraniliprole (40 g a.i. ha⁻¹, 100 mL ha⁻¹), respectively.

The insecticides/dosages were diluted in water and anionic surfactant polyoxyethylene alkyl phenol ether (0.15 mL L⁻¹) (Haiten 200, Arysta, Brazil). The control treatment was composed of water + surfactant (0.15 mL L⁻¹). Prior to spraying (pre-evaluation), the number of test species *C. sanguinea*, *O. insidiosus* and *C. flavipes* per plant were counted on the five plants in the center of each parcel, using a white tray following (Bacci et al., 2007). The trays were positioned on the apical, median and basal regions of each plant, with their leaves shaken over the tray. Test species were counted as they fell onto the tray. After the pre-evaluation the insecticides and control plots were sprayed with a 20 L CO₂ backpack sprayer at a constant pressure of 25 psi and a spray volume of 500 L ha⁻¹. A directed spray (~75% band, with rate adjusted for band) was delivered through three nozzles (TX-18) per bed. Twenty minutes after spraying, each treatment was evaluated, as noted above, on the day of spraying, 7, 14 and 21 d after spraying.

2.6. Data analysis

2.6.1. Filter paper lethal toxicity tests

Concentration-mortality results were subjected to probit analysis, correcting the data for natural mortality (Proc Probit) (SAS Institute, 2002). The relative toxicity (RT_{20 or 50}) was calculated by higher LC_{20 or 50} value of insecticide (least toxic)/lower LC_{20 or 50} value (most toxic) of the other insecticides (Adapted from Bacci et al., 2001).

2.6.2. Feeding after contact with insecticides and reproduction

Prey consumption and behavioral reproduction data were analyzed by one-way univariate ANOVA and the means were separated using the Scott-Knott test ($\alpha = 0.05$).

2.6.3. Repellency (paired choice bioassay)

The number of test species on the control half of the leaf and the half treated with the insecticide was compared using the paired *t*-test ($\alpha = 0.05$).

2.6.4. Toxicity under field conditions

The data on the number of predators were analyzed by one-way univariate ANOVA (interactions: treatments × days after spraying) and the means were separated using the Tukey test ($\alpha = 0.05$).

For all analyses, the assumptions of normality and homogeneity of variance were checked (Proc Univariate) using SAS System software (SAS Institute, 2002) with no transformation being necessary.

Table 2
Relative toxicity of insecticides on test species *Cycloneda sanguinea*, *Orius insidiosus* and *Chauliognathus flavipes*.

Insectic. ^a	Test species ^b				
	<i>Cycloneda sanguinea</i>				
	Slope	LC ₅₀ (mg a.i. cm ⁻²) ^c	RT ₅₀ ^d	LC ₂₀ (mg a.i. cm ⁻²) ^c	RT ₂₀ ^d
Chlorpyrifos	1.13 ± 0.35	0.14(0.11–0.15)	14.93	0.04(0.11–0.15)	39.47
Chlorantraniliprole	1.02 ± 0.31	2.03(1.90–2.08)	1.00	1.50(1.42–1.55)	1.00
Deltamethrin	4.11 ± 1.71	0.29(0.25–0.41)	7.13	0.04(0.04–0.06)	34.34
Acephate	5.02 ± 1.06	0.17(0.14–0.18)	12.17	0.03(0.01–0.10)	54.35
Imidacloprid	1.22 ± 0.09	0.76(0.71–0.78)	2.66	0.11(0.02–0.46)	13.38
Bifenthrin	1.45 ± 0.11	0.28(0.26–0.32)	7.13	0.04(0.04–0.32)	35.63
Thiamethoxam	1.01 ± 0.05	0.42(0.40–0.46)	4.89	0.26(0.16–0.58)	3.60
<i>Orius insidiosus</i>					
Chlorpyrifos	1.32 ± 0.21	0.20(0.16–0.23)	20.34	0.00(0.00–0.05)	327.3
Chlorantraniliprole	1.01 ± 0.35	4.05(3.95–4.07)	1.00	0.93(0.71–0.79)	1.00
Deltamethrin	2.10 ± 1.72	0.25(0.24–0.26)	16.05	0.02(0.00–0.10)	48.29
Acephate	7.14 ± 1.07	0.03(0.02–0.05)	110.4	0.00(0.00–0.10)	1178
Imidacloprid	1.39 ± 1.22	0.08(0.08–0.09)	48.29	0.00(0.00–0.01)	736.4
Bifenthrin	1.17 ± 0.49	0.21(0.18–0.35)	19.57	0.02(0.00–0.05)	56.10
Thiamethoxam	0.83 ± 0.04	0.38(0.32–0.56)	10.64	0.04(0.00–0.11)	25.95
<i>Chauliognathus flavipes</i>					
Chlorpyrifos	1.42 ± 0.85	0.36(0.32–0.38)	23.92	0.16(0.08–0.27)	4.85
Chlorantraniliprole	1.01 ± 0.02	8.60(8.35–8.92)	1.00	0.79(0.65–0.90)	1.00
Deltamethrin	12.10 ± 1.72	0.25(0.24–0.26)	34.12	0.09(0.24–0.26)	8.29
Acephate	27.14 ± 1.07	0.04(0.02–0.06)	234.8	0.01(0.00–0.02)	135.2
Imidacloprid	11.39 ± 1.22	0.08(0.08–0.09)	102.6	0.01(0.00–0.05)	138.9
Bifenthrin	1.43 ± 0.41	0.30(0.26–0.36)	28.31	0.01(0.00–0.05)	135.2
Thiamethoxam	1.31 ± 0.52	0.47(0.44–0.51)	18.15	0.01(0.00–0.02)	416.7

Mortality after 36 h insecticides-contact exposure period (filter paper-dip method).

a.i. = active ingredient.

^a Number of insecticides = 7.

^b Adult phase exposure.

^c Probit analysis (Chi-square, $\alpha = 0.05$).

^d RT_x (relative toxicity): higher LC_x value of insecticide/lower LC_x value of other insecticide; x = 50 or 20.

Table 3
Effects on reproductive behavior of the three test species caused by sublethal concentrations (LC₂₀) of the seven insecticides.

Insecticide ^a	Reproductive parameters ¹			
	Sexual rate (female/female + male) ^b	Fecundity (eggs/female) ^c	Viability (%) ^d	Number of offspring/female
<i>Cycloneda sanguinea</i>				
Chlorpyrifos	0.61 ± 0.00a	157.15 ± 0.25a	75.36 ± 3.32a	119.02 ± 1.00a
Chlorantraniliprole	0.44 ± 0.01a	171.43 ± 1.00a	68.52 ± 1.17a	110.05 ± 0.04a
Deltamethrin	0.55 ± 0.01a	185.67 ± 1.77b	55.47 ± 0.87b	88.77 ± 0.04b
Acephate	0.60 ± 0.01a	160.40 ± 1.65a	67.41 ± 3.14a	115.46 ± 0.01a
Imidacloprid	0.64 ± 0.01a	161.00 ± 1.00a	69.00 ± 7.87a	118.00 ± 1.00a
Bifenthrin	0.63 ± 0.00a	163.10 ± 1.00a	72.12 ± 2.74a	119.95 ± 0.01a
Thiamethoxam	0.44 ± 0.00a	191.11 ± 1.00c	88.44 ± 2.11c	119.97 ± 1.08a
Control	0.55 ± 0.02a	168.45 ± 1.12a	75.52 ± 1.04a	116.02 ± 1.00a
<i>Orius insidiosus</i>				
Chlorpyrifos	0.61 ± 0.00a	112.00 ± 12.11a	15.16 ± 3.32a	118.77 ± 1.30a
Chlorantraniliprole	0.44 ± 0.01a	141.10 ± 12.31b	68.74 ± 2.01b	117.01 ± 1.00a
Deltamethrin	0.55 ± 0.05a	166.05 ± 10.01d	55.47 ± 0.11c	115.37 ± 0.10a
Acephate	0.60 ± 0.01a	112.00 ± 10.12a	13.41 ± 1.17a	72.11 ± 0.11b
Imidacloprid	0.64 ± 0.01a	111.07 ± 11.87a	54.00 ± 1.03c	116.10 ± 0.01a
Bifenthrin	0.63 ± 0.00a	100.32 ± 13.45a	60.12 ± 1.00b	119.31 ± 0.00a
Thiamethoxam	0.44 ± 0.00a	131.09 ± 11.23c	65.44 ± 3.01b	119.21 ± 0.01a
Control	0.55 ± 0.00a	145.31 ± 13.01b	70.12 ± 4.04b	110.38 ± 0.02a
<i>Chauliognathus flavipes</i>				
Chlorpyrifos	0.50 ± 0.03a	82.14 ± 9.07a	41.10 ± 0.02a	73.49 ± 1.17c
Chlorantraniliprole	0.50 ± 0.02a	203.30 ± 13.00f	78.37 ± 0.37d	129.71 ± 1.30a
Deltamethrin	0.48 ± 0.00a	95.11 ± 11.91c	51.48 ± 0.17a	75.37 ± 0.00c
Acephate	0.64 ± 0.01a	122.50 ± 10.74d	37.93 ± 0.98b	20.11 ± 0.11b
Imidacloprid	0.55 ± 0.00a	111.07 ± 05.99b	14.66 ± 0.97c	126.41 ± 0.06a
Bifenthrin	0.61 ± 0.01a	127.39 ± 14.41d	38.00 ± 0.57b	129.47 ± 0.01a
Thiamethoxam	0.50 ± 0.01a	144.00 ± 21.10e	77.08 ± 0.08d	129.09 ± 0.96a
Control	0.54 ± 0.01a	215.97 ± 11.40f	79.11 ± 0.09d	131.61 ± 0.07a

¹ Values in the same column (comparing each test species) with different lowercase letters show significant differences at $P < 0.05$ level by the Scott-Knott test. Contact 60 h exposure period (topical application - 1 μ L of insecticide per insect). Adult phase exposure to LC₂₀ to each insecticide determined in Table 2.

^a Number of insecticides = 7.

^b Number of insects (per treatment).

^c Total neonates/total neonates plus all unhatched eggs.

^d Percentage of eggs yielding viable neonates.

3. Results

3.1. Lethal toxicity tests

In general, the insecticides chlorpyrifos and acephate were the most toxic, since they showed the lowest required concentrations to kill 50% of the test organisms (the LC_{50} between the three test species ranged from 0.14 to 0.36 mg a.i. cm^2 and 0.03–0.17 mg a.i. cm^2), respectively. On the other hand, chlorantraniliprole was the least toxic (ranging from 2.03 to 8.60 mg a.i. cm^2).

The natural mortality observed in the control treatments for three test species was below 1% and was used to correct for insecticide mortality. Based on lethal concentration (50%) and confidence interval of the bioassays of concentration-mortality, the insecticide of the group of the anthranilamides (chlorantraniliprole) was considered the least toxic to the test species *C. sanguinea*, *O. insidiosus* and *C. flavipes*. The organophosphates, chlorpyrifos and acephate insecticides were the most toxic, with the chlorpyrifos being more toxic to *C. sanguinea* and acephate more toxic to *O. insidiosus* and *C. flavipes*. The toxicity of the acephate was also shown by the higher slopes of the curves of the test species *C. sanguinea* (5.02), *O. insidiosus* (7.14) and *C. flavipes* (27.14) (Table 2). Chlorpyrifos showed a relative toxicity to *C. sanguinea* of 14.93 times greater than the insecticide chlorantraniliprole. Since acephate showed relative toxicity of 110.45 and 234.77 times higher than that of chlorantraniliprole to the test species *O. insidiosus* and *C. flavipes* (Table 2). The insecticide imidacloprid was 102.63 times more toxic to the test species *C. flavipes* than chlorantraniliprole.

3.2. Feeding after contact with insecticides

Significant differences were observed in the feeding behavior of the test species *C. sanguinea* ($F_{2,216} = 314.2$, $P < 0.001$), *O. insidiosus* ($F_{2,216} = 33.12$, $P < 0.001$) and *C. flavipes* ($F_{2,216} = 120.3$, $P < 0.001$) when in contact with the lethal concentration (20%) of the seven insecticides. Differences were also observed in the interactions between the insecticides and the species of test species ($F_{14,216} = 210.5$, $P < 0.001$).

Chlorantraniliprole was the only insecticide that did not affect the feeding behavior of any test species, with prey consumption being similar to the control. Bifenthrin did not alter the feeding behavior of *C. sanguinea*. The number of prey items consumed by *C. sanguinea* was reduced by 74, 90, 78, 50, 12 and 36% after exposure to chlorpyrifos, deltamethrin, acephate, imidacloprid, bifenthrin and thiamethoxam insecticides, respectively (Fig. 1A). *O. insidiosus* was reduced by 90, 97, 83, 57, 67 and 83% (Fig. 1B) and *C. flavipes* reduced its feeding by 52, 76, 98, 76, 57 and 81% (Fig. 1C).

3.3. Repellency

Our results showed that all insecticides induced repellency to at least one test species, with *C. sanguinea* being repelled by all insecticides, with 75–95% variation in repellence. For *O. insidiosus* and *C. flavipes* variation in repellence was 60–100% and 55–85%. The insecticides bifenthrin, imidacloprid and acephate functioned as repellents to all test species, since treatments with these insecticides showed significantly lower numbers of *C. sanguinea*, *O. insidiosus* and *C. flavipes* adults than the control. The acephate repelled 100% of *O. insidiosus* ($t = 12.78$, $df = 19$, $P < 0.001$). On the other hand, the insecticide thiamethoxam did not show repellency to *O. insidiosus* ($t = 0.25$, $df = 19$, $P = 0.250$), nor was chlorantraniliprole repellent to *O. insidiosus* ($t = 1.05$, $df = 19$, $P = 0.131$) or *C. flavipes* ($t = 0.74$, $df = 19$, $P = 0.195$) (Fig. 2).

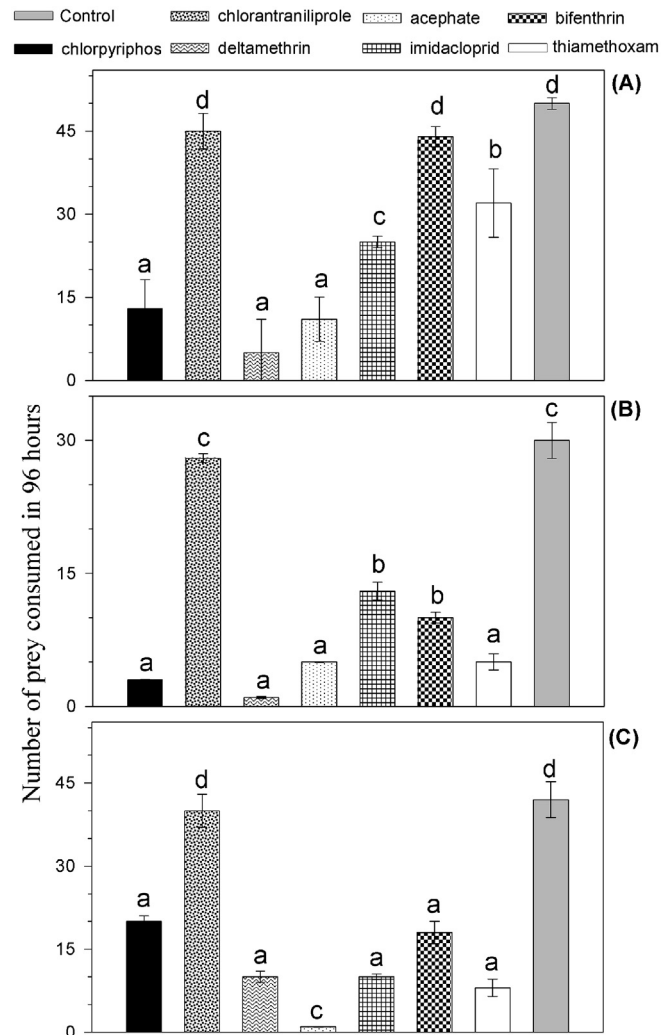


Fig. 1. Number of prey consumed per test species *Cycloneda sanguinea* (A), *Orius insidiosus* (B) and *Chauliognathus flavipes* (C) after 96 h of exposure to insecticides (LC_{20}). Columns with the same letters did not differ significantly when comparing insecticides ($P = 0.05$, Scott-Knott test). Bioassay of feeding after contact with insecticides; adult phase exposure to LC_{20} for each insecticide determined in Table 2; Number of insecticides = 7; n = number of prey per treatment.

3.4. Reproduction

Of the insecticides tested, contact exposure to seven substances affecting fecundity of at least one test species, with thiamethoxam affecting *C. sanguinea* and chlorpyrifos, deltamethrin, acephate, bifenthrin, imidacloprid and thiamethoxam affecting *O. insidiosus* and *C. flavipes*. Contact exposure to chlorantraniliprole did not significantly affect test species fecundity at LC_{20} . The insecticides deltamethrin (on *C. sanguinea*), acephate (on *O. insidiosus* and *C. flavipes*) and chlorpyrifos (*C. flavipes*) had significant effects on the number of offspring/female (Table 3).

Extreme effects on the reproductive parameter (viability) were observed for chlorpyrifos, deltamethrin, acephate, bifenthrin and imidacloprid insecticides. Acephate reduced the viability of *O. insidiosus* eggs by 13.4% when adults were exposed to this insecticide. In addition, imidacloprid reduced the viability of *C. flavipes* by 14.7%. A curious response was observed regarding the effect of deltamethrin on *C. sanguinea* and *O. insidiosus* test species, which showed fecundity of 185.7 and 166.0. These values were significantly higher than those of the controls (1.10 and 1.14 times)

(Table 3).

3.5. Toxicity under field conditions

Significant differences were observed between the number of test species in each type of insecticide (treatment) ($F_{7,62} = 78.33$, $P < 0.001$), between the evaluation time ($F_{3,62} = 93.86$, $P < 0.001$) and treatment \times time interaction ($F_{21,62} = 61.02$, $P < 0.001$). In a general way, of the six insecticides sprayed in field conditions, only chlorantraniliprole did not affect the test species. The insecticides from the organophosphate group (acephate and chlorpyrifos) and the pyrethroids (bifenthrin and deltamethrin) were the insecticides that most reduced test species densities. In general the test species were reduced in the days after spraying, with populations of *O. insidiosus* and *C. flavipes* reduced by 100% (Fig. 3 B,C). The populations did not increase again until 21 d after spraying (Fig. 3). Population growth occurred in the control group and the populations exposed to the insecticide chlorantraniliprole (Fig. 3). The insecticides chlorpyrifos and chlorantraniliprole showed higher concentrations in the field than the laboratory LC₅₀. Moreover other insecticides had the lowest concentrations of the laboratory (Tables 1 and 2).

4. Discussion

4.1. Lethality in the laboratory

We assessed the acute insecticide toxicity response of adults (*O. insidiosus*, *C. sanguinea* and *C. flavipes*) to determine the safety of new and old insecticides registered for pest control in Brazil. In a general manner the insecticides that showed high toxicity to the test species in laboratory conditions may or not cause toxic effects in field conditions. This is because the insecticide may degrade in field conditions, reducing the concentration and toxicity to the insect (Eijaza et al., 2015). On the other hand, the insecticides that did not show toxicity to the test species in laboratory conditions were not expected to be toxic in field conditions, since insecticides suffer greater chemical and biological degradation in the field than in the laboratory (Byerlee et al., 2009). Moreover, because the insecticide spray concentrations are higher in the field (see Table 1) than the LC₂₀ and LC₅₀ (see Table 2) of the natural enemies in the laboratory, the insecticides affected high of the natural enemies in the field.

Based on our study the most promising insecticide was the chlorantraniliprole, which showed lower toxicity in laboratory (>LC₅₀) and field conditions at the recommended dosages. The highest concentration needed to kill 50% of the population was observed for *C. flavipes* (8.60 mg i.a. cm²), a value ~46511 times lower than the field dose (4.0×10^5 mg a.i. cm⁻²). This trait was expected for chlorantraniliprole based on recent toxicity studies with predatory bugs and mites (Dinter et al., 2008; Preetha et al., 2010) because of the high affinity of chlorantraniliprole towards Lepidoptera ryanodine receptors (Nauen, 2006). Although there are no studies on this insecticide with *O. insidiosus*, there have been studies on other Hemiptera, e.g. Biondi et al. (2012) who did not observe chlorantraniliprole toxicity in *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae), as well as Castro et al. (2013) who also did not detect toxicity of this insecticide to *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stal) (Heteroptera: Pentatomidae) and Martinou et al. (2014) with *Macrolphus pygmaeus* (Hemiptera: Miridae). Castro et al. (2013) observed that chlorantraniliprole was the least toxic and most selective insecticide to *Podisus nigrispinus* with these test species experiencing mortalities of less than 10% when exposed to 133 $\mu\text{g a.i. mL}^{-1}$, the recommended field concentration, for a period of 72 h. There are no studies of this

insecticide on *C. sanguinea* and *C. flavipes*, but it is expected that there would be little effect due to the specificity of the insecticide.

4.2. Sublethality in the laboratory

With the exception of the insecticide chlorantraniliprole, all insecticides negatively affected feeding and reproductive behavior or repelled the test species at sublethal concentrations. Sublethal insecticide doses may reduce predator efficiency (Roger et al., 1994), disrupt prey defenses and reduce the probability of encounter (by reducing predator and prey mobility) (Jackson and Ford, 1973).

The lower the feeding capacity and the higher the repellence of the test species caused by the insecticides, the lower the rate of predation, which consequently reduces the rate (Desneux et al., 2007). Studies with organophosphates, pyrethroids and neonicotinoids have been done to evaluate the effects on feeding and reproductive behavior (Desneux et al., 2007; Casida and Durkin, 2013). These studies often show sublethal effects of insecticides on test species. Singh et al. (2004) verified that sublethal effects of organophosphate insecticide residues may result in an immediate reduction in the efficiency of coccinellids to locate their prey. These authors showed that *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) spend proportionately less time on the apex of the plant compared with the control treatment. Martinou et al. (2014) observed that the insecticide chlorantraniliprole did not affect the predation capacity of the predator *Macrolphus pygmaeus* (Rambur) (Hemiptera: Miridae).

The pyrethroid bifenthrin and chlorpyrifos are probably the best known repellent insecticides studied in this work. Repellency associated with pyrethroids and chlorpyrifos has long been considered a behavioral adaptation for reducing the risk of exposure. Insecticides in these groups may irritate or repel more predators by acting directly on the central or peripheral nervous system (Rinkevich et al., 2013; NPIC, 2014).

On the other hand, deltamethrin increased the fertility rate of *C. sanguinea* and *O. insidiosus*. This increase may be due to the phenomenon of hormesis (Hunt et al., 2011), which has been observed with various insects (McClure et al., 2014). Particularly, pyrethroids showed hormesis in pests and predators (Forbes, 2000; Cordeiro et al., 2013; Zanoncio et al., 2013). This suggests an adaptive strategy to allocate resources between reproduction and longevity necessary to improve maintenance during periods of low prey density, quality and the presence of stressor agents (Molina-Rugama et al., 1998; Wittmeyer et al., 2001). Neonicotinoids exhibit a variety of lethal and sublethal effects on behavior such as feeding, oviposition, and fecundity in arthropods (Casida and Durkin, 2013).

4.3. Lethality in the field

The recommended doses of the pyrethroids and organophosphates were those that most affected the test species, with population reductions up to 100%. This shows that these insecticides would not be good candidates since they reduce the populations of natural enemies (test species) and fail to control pest species in Brazil (Silva et al., 2010; Gontijo et al., 2014). In general the field doses of insecticides were high than laboratory doses (See Tables 1 and 2).

Organophosphate insecticides are generally regarded as highly toxic to natural enemies. This was confirmed for the organophosphate chlorpyrifos and acephate in previous studies with the test species families (Bacci et al., 2009; Cordeiro et al., 2010). Compared to laboratory data, we observed that the field doses were higher. The high toxicity of the organophosphates chlorpyrifos and

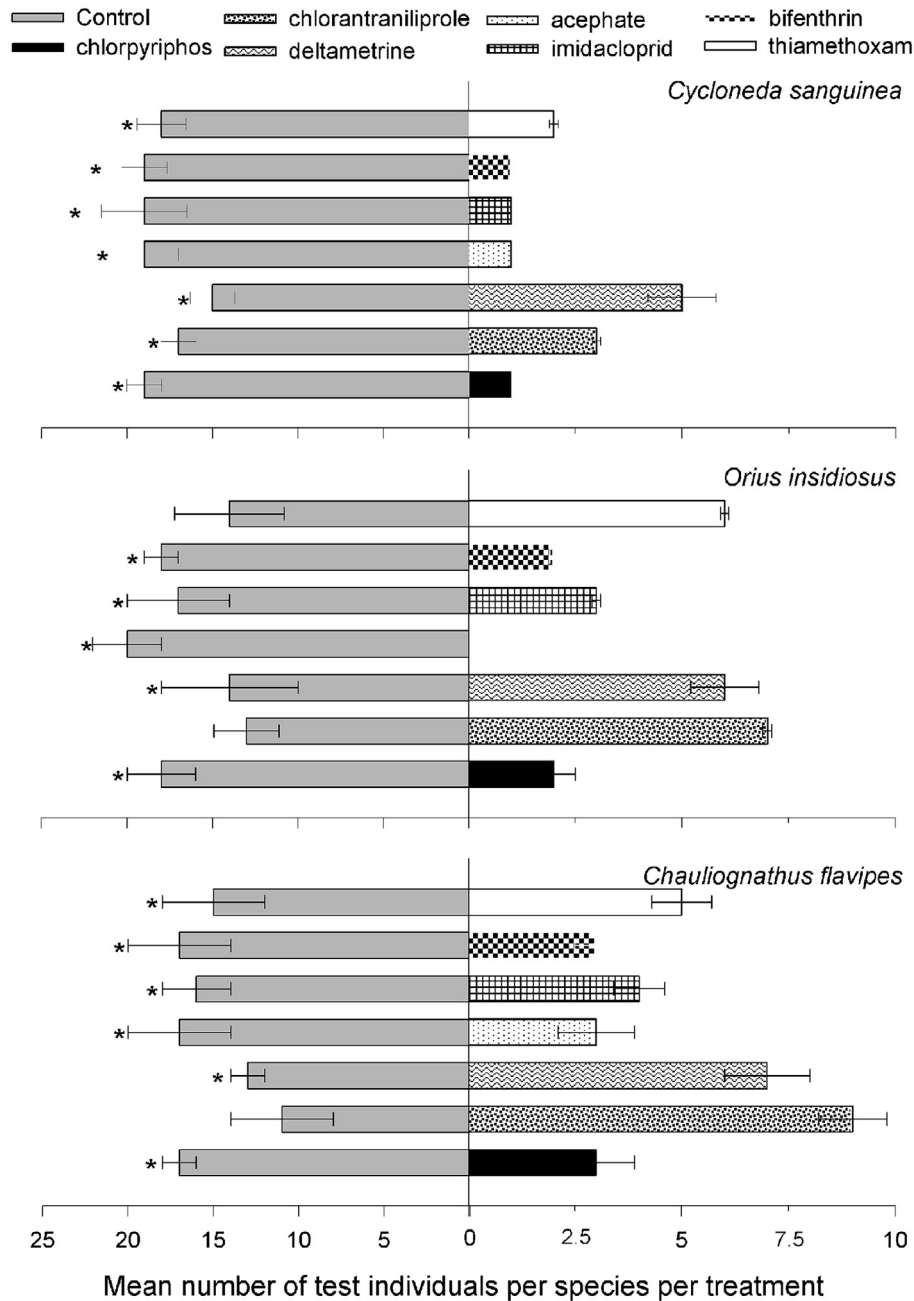


Fig. 2. Response (number of test species) of *Cycloneda sanguinea*, *Orius insidiosus* and *Chauliognathus flavipes* on filter paper treated with insecticide and control. The left side of the figure represents the control half of the filter paper discs and the right side the treated half of the filter paper discs. Means \pm SE differed significantly according to the t-test of paired series at the 5% significance level. n = number of prey per treatment; evaluation 15 min after adults were released.

acephate to all test species is possibly associated with the low solubility in water and high molecular weight of these molecules (0.91 g L^{-1} at $25 \text{ }^\circ\text{C}$ and $183.16 \text{ g mol}^{-1}$) (Berg et al., 2003). These properties of low solubility and high molecular weight are important in insecticide toxicity because they have higher affinity with the wax-based compounds show in the cuticle of insects (Fraenkel and Rudall, 1940; Vincent and Wegst, 2004). Additionally, it is probable that the test species have low metabolism by enzymatic detoxification dependent on monooxygenases such as P-450, involving the metabolism of the organophosphates. Recently, field populations of *Eriopis connexa* Germar (Coleoptera: Coccinellidae) in contact with neurotoxic insecticides were classified as resistant and susceptible. The probable cause of the resistance was

associated to the activities of cytochrome P450-dependent microsomal (Rodrigues et al., 2013, 2014). Other studies on *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *Hippodamia convergens* (Guérin-Méneville) have been reported (Sayyed et al., 2010; Rodrigues et al., 2013).

The intermediary response of the toxicity (see LC_{50} in Table 2) of the pyrethroids (bifenthrin and deltamethrin) and neonicotinoids (thiamethoxam) to the test species does not mean their toxicity is low, since they differed significantly in the confidence interval of the relative toxicity (50%) of chlorantraniliprole. Pyrethroid insecticides are generally regarded as very toxic to natural enemies (e.g., Cordeiro et al., 2010; Rodrigues et al., 2013). The two active ingredients (bifenthrin and deltamethrin) show similar toxicity to

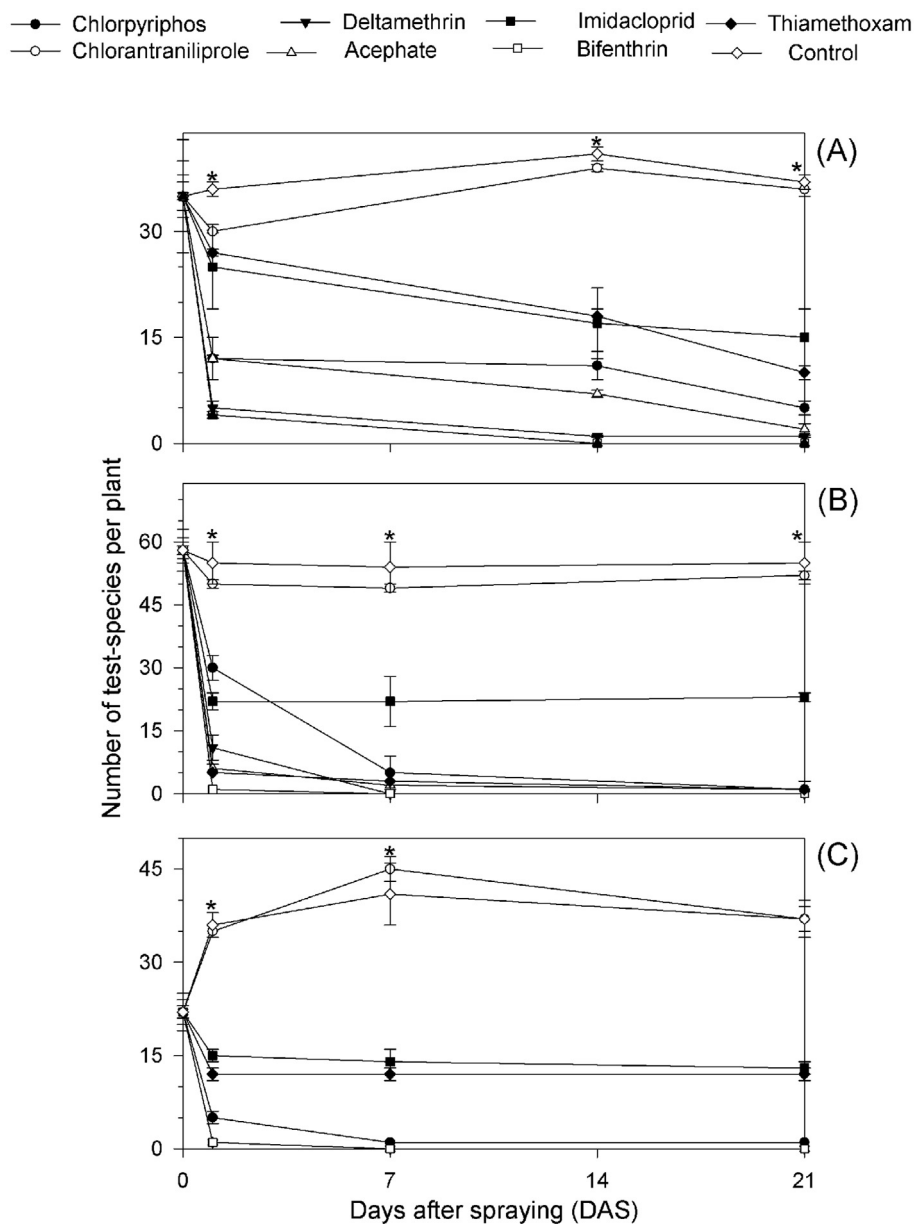


Fig. 3. Bioassay field experiment with mean \pm SD of number of test species *Cycloneda sanguinea* (A), *Orius insidiosus* (B) and *Chauliognathus flavipes* (C) at 0, 1, 7 and 21 d after insecticide applications. Asterisks show significant differences among treatments and time at the 95% confidence interval.

test species. In sweet corn, the densities of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) larvae in plots treated with bifenthrin were low compared to the control (Galvan et al., 2005). Ahmad et al. (2011) observed high *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) mortality to the pyrethroid deltamethrin.

We tested the maximum exposure of test species to the neonicotinoid insecticides and verified their low toxicity. The low toxicity of thiamethoxam to test species could be the consequence of their high solubility in water (4.10 g L^{-1} at $25 \text{ }^\circ\text{C}$) (Berg et al., 2003). This may be true because studies have shown that test species mortality can result from contact with systemic insecticides, consumption of insecticide-contaminated leaf tissue, or both (Ridgway et al., 1967; Hough-Goldstein and Whalen, 1993).

In conclusion, the insecticide chlorantraniliprole was the most promising compound for use in tomato caterpillars while having

low lethal and sublethal effects on the test species *C. sanguinea*, *O. insidiosus* and *C. flavipes*. Reduced feeding, repellency and reproduction were observed in individuals treated with organophosphates (acephate and chlorpyrifos) and pyrethroids (bifenthrin and deltamethrin) and these were the insecticides that most reduced the densities of test species in field conditions. Deltamethrin induced hormesis in *C. sanguinea* and *O. insidiosus*. In our study the hormetic effect caused by the exposure of the test species to low (sublethal) levels of pesticide, heat or nutritional stress may also have potential use for enhancing the yield and efficacy of natural enemies such as predators, parasitoids or insect pathogens. Hormesis might result in economic and performance gains in culturing natural enemies, reflecting the observed yield increases in plants exposed to low levels of stress (Guedes and Cutler, 2014). Field treatments with chlorantraniliprole had no effect on mortality. We suggest that the inclusion of organophosphates and

pyrethroids on predators integrated pest management programs be evaluated with caution, since the product should be selective of natural enemies to be effective against pests.

Acknowledgments

We thank the Minas Gerais State Foundation for Research Aid (FAPEMIG) (CAG - APQ-03009-13), the CAPES Foundation (Brazilian Ministry of Education), and the National Council for Scientific and Technological Development (304198/2015-3) (CNPq; Brazilian Ministry of Science and Technology) for scholarships and financial support provided. We also Dr. Paulo S. F. Ferreira and Dr. Ayr de Moura Bello for identifying natural enemies.

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