Survival and behavior of the insecticide-exposed predators *Podisus nigrispinus* and *Supputius cincticeps* (Heteroptera: Pentatomidae)

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HIGHLIGHTS

- Deltamethrin, methamidophos and spinosad caused 100% mortality of predators.
- Chlorantraniliprole showed lower predator toxicity and higher selectivity.
- All of the insecticides sparked changes in the predator (walking) behavior.
- Insecticide repellence was not observed for any compounds tested.
- Chlorantraniliprole use is advisable for IPM programs.

ARTICLE INFO

ABSTRACT

Pentatomid stinkbugs are important predators of defoliating caterpillars in agricultural and forestry systems, and knowledge of the impact of insecticides on natural enemies is important information for integrated pest management (IPM) programs. Thus, we assessed the toxicity and behavioral sublethal response of the predators *Podisus nigrispinus* and *Supputius cincticeps* exposed to deltamethrin, methamidophos, spinosad and chlorantraniliprole, insecticides commonly used to control the velvetbean caterpillar (*Anticarsia gemmatalis*) in soybean crops. With the exception of deltamethrin for *S. cincticeps*, all insecticides showed higher acute toxicity to the prey than to these natural enemies providing effective control of *A. gemmatalis*. The recommended field concentration of deltamethrin, methamidophos and spinosad for controlling *A. gemmatalis* caused 100% mortality of *P. nigrispinus* and *S. cincticeps* nymphs. Chlorantraniliprole was the less toxic and the most selective insecticide to these predators resulting in mortalities of less than 10% when exposed to 10× the recommended field concentration for a period of 72 h. Behavioral pattern changes in predators were found for all insecticides, especially methamidophos and spinosad, which exhibited irritability (i.e., avoidance after contact) to both predator species. However, insecticide repellence (i.e., avoidance without contact) was not observed in any of the insects tested. The lethal and sublethal effects of pesticides on natural enemies is of great importance for IPM, and our results indicate that substitution of pyrethroid and organophosphate insecticides at their field rates by chlorantraniliprole may be a key factor for the success of IPM programs of *A. gemmatalis* in soybeans.

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1. Introduction

Insecticide selectivity and impact on natural enemies are key components of Integrated Pest Management (IPM) programs (Metcalf, 1980; Hardin et al., 1995; Desneux et al., 2007). Chemical control is the most common method used to control pests (Cooper and Dobson, 2007; Song and Swinton, 2009) and its use has increased in various cultures, notably in developing countries, despite of a few exceptions (e.g. China) due to increased use of transgenic crops (Song and Swinton, 2009; Meissle et al., 2010; Lu et al., 2012; Pedlowski et al., 2012). Simultaneously, changes in societal attitude has triggered the search for safer pesticides to humans and the environment, resulting in the development of compounds more specific to the target pest, i.e. for non-target organisms

(Matsumura, 2004; Cordova et al., 2006; Nicholson, 2007). However, problems related to pollution by pesticides and overuse of these chemicals still remain. Historically, crop protection has often resulted in the application of pesticides harmful to natural enemies (Wilson and Tisdell, 2001; Desneux et al., 2007). IPM aims to reduce the status of pests to tolerable levels with the use of effective, economically sustainable and ecologically sound management (Van Lenteren and Woets, 1988). Although pesticide use remains an important IPM tactic, efforts have been made in the search for compounds with reduced impact on natural enemies and other non-target arthropods. Thus, studies assessing lethal and sublethal effects of pesticides on these organisms are increasingly performed, though primarily at the population level (Stark and Banks, 2003; Desneux et al., 2007; Stark et al., 2007; Zanuncio et al., 2011; Castro et al., 2012; Seagraves and Lundgren, 2012; Biondi et al., 2012b). Exposure to a particular product may trigger adverse effects not necessarily resulting in the death of individuals (Desneux et al., 2007). These sublethal effects may comprise physiological parameters such as development, longevity and fecundity, as well as behaviors involved in mobility, foraging for hosts (or prey) and mates (Desneux et al., 2004a,b; Kim et al., 2006; Harwood et al., 2007; Suma et al., 2009; Evans et al., 2010; Cabral et al., 2011; Caballero-López et al., 2012; Stara et al., 2011; He et al., 2012).

Arthropod predators are important in crops due to the ability to control phytophagous insects and mites (Symondson et al., 2002). Species of the subfamily Asopinae (Pentatomidae) are important predators of defoliating caterpillars (Zanuncio et al., 2003; Castro et al., 2012). These natural enemies can achieve significant populations feeding on other prey and plants before the arrival of pests (Zanuncio et al., 2004; Desneux and O'Neil, 2008; Holtz et al., 2011). They also display generalist behavior (Shapiro and Legaspi, 2006) with adaptation to different temperatures and prey (Vivan et al., 2003; Legaspi, 2004; Silva et al., 2012) and relative tolerance to insecticides (Smagghe and Degheele, 1995; Zanuncio et al., 2011; Castro et al., 2012), which emphasizes the importance of these for potential success of IPM programs (Zanuncio et al., 2008; Pires et al., 2011).

Anticarsia gemmatalis Hübner (Lepidoptera: Noctuidae) is one of the major lepidopteran pests of soybeans occuring from Argentina to the United States, causing serious defoliation on plants during their vegetative and reproductive stages (Walker et al., 2000; Homrich et al., 2008). The use of insecticides is still one of the main methods for controlling this pest (Silva et al., 2011) and research is carried out to identify compounds with low toxicity to natural enemies in IPM programs of *A. gemmatalis*. We assessed the acute toxicity and behavioral sublethal response of the predators *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stal) (Heteroptera: Pentatomidae) exposed to deltamethrin, methamidophos, spinosad and chlorantraniliprole. These insecticides are used for *A. gemmatalis* control and this study may help optimizing combined use of pesticides and natural enemies for management of *A. gemmatalis,* while exhibiting low toxicity to natural enemies.

2. Materials and methods

2.1. Insects

The predators *P. nigrispinus* and *S. cincticeps* and the prey *A. gemmatalis* were obtained from mass-reared cultures from the Laboratory of Biological Control of Insects (LCBI) of the Institute of Biotechnology applied to Agriculture (BIOAGRO), at the Federal University of Viçosa (UFV), Viçosa, Minas Gerais State, Brazil. These natural enemies are reared with pupae of the yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) under controlled environmental conditions (25 ± 2 °C, 70 ± 5% relative humidity,

and 12:12 light:dark photoperiod) (Molina-Rugama et al., 1997; Zanuncio et al., 2000). Yellow mealworm adults and larvae are reared on a plastic tray containing wheat flour mixed with yeast ($\approx$5%) and vegetables such as carrot, sweetpotato, and cassava, as food and moisture supplied once a week. More details on producing yellow mealworms can be obtained in Zamperline et al. (1992). Caterpillars of *A. gemmatalis* are reared on artificial diet (Greene et al., 1976) and their adults in wooden cages ($30 \times 30 \times 30$ cm) with screened sides, glass covers and fed cotton soaked in nutrient solution at the bottom of the cages. Nymphs of *P. nigrispinus* and *S. cincticeps* and larvae of *A. gemmatalis* larvae were observed daily to obtain third-instar insects for use in the bioassays.

2.2. Insecticides

All of the insecticides used are registered for controlling *A. gemmatalis* in Brazilian soybean fields (Agrofit, 2012). The insecticides used and their respective commercial formulations were: the pyrethroid deltamethrin (Decis® 25 EC; 25 g a.i. L^{-1}; Bayer CropScience Ltd.; São Paulo-SP), the organophosphate methamidophos (Tamaron® BR SC; 600 g a.i. L^{-1}; Bayer CropScience Ltd.; Belford Roxo-RJ), the diamide chlorantraniliprole (Premio® CS; 200 g a.i. L^{-1}; DuPont Brasil S.A.; Barra Mansa-RJ) and the spinosyn spinosad (Tracer® 480 CS; 480 g a.i. L^{-1}; Dow AgroSciences Industrial Ltd.; São Paulo-SP).

2.3. Concentration-mortality bioassays

The concentration-mortality bioassays were carried out using Petri dishes (9.0 cm diameter $\times$ 2.0 cm high) with the bottom completely covered with soybean leaves of the cultivar "BRSMT pintado" treated with insecticide solutions. For each treatment, the soybean leaves were immersed for five seconds at different concentrations of each insecticide solution (diluted in water) and the leaves were let to dry in shade for an hour before placement in the Petri dishes (Castro et al., 2012). Each Petri dish received ten third-instar larvae of *A. gemmatalis* or ten third-instar nymphs of *P. nigrispinus* or *S. cincticeps*. Bioassays were established following a completely randomized design with five to eight concentrations and six replicates. The concentrations used were established through preliminary bioassays with a 10-fold range of dilutions for each insecticide and species to allow recognition of the concentration range leading to mortality variation between 0% and 100%. Mortality was assessed after 72 h of exposure and the insects were considered dead if they did not move when prodded with a fine hair brush. Predators were not fed during the exposure to the insecticide in this bioassay since they can survive to over 14 d without prey as a food source (Lemos et al., 2001).

2.4. Time-mortality bioassays under insecticide field rates

The acute (lethal) toxicity towards predatory stinkbugs of the maximum recommended insecticide concentrations for the control of *A. gemmatalis* (chlorantraniliprole-13.3 μg a.i mL^{-1}, deltamethrin-50 μg a.i. mL^{-1}, spinosad-240 μg a.i. mL^{-1} and methamidophos-1500 μg a.i. mL^{-1}) was estimated using third-instar nymphs of *P. nigrispinus* and *S. cincticeps*. Ten nymphs of each species were placed over the insecticide-impregnated filter paper glued (with synthetic white water-based glue resin) to the bottom of a Petri dish (9 cm diameter $\times$ 2 cm high), whose inner walls were covered with Teflon® PTFE (DuPont, Wilmington, DE, USA) to prevent insect escape. The filter paper disc was considered treated when soaked for 5 s with 1 mL of solution corresponding to each recommended field concentration of insecticide. Five replicates were used for each combination of insecticide and predator species, in addition to a control treatment were only water (distilled and deionized)

was applied to the filter papers. Insect mortality was observed every 30 min during the initial 24 h exposure and at 5 h intervals afterwards until the death of all insects or until they reached the adult stage. *Tenebrio molitor* pupae were provided *ad libitum* to the predatory stinkbug nymphs throughout the bioassays. The nymphs were recorded as dead if they were unable to move when dorsally prodded with a fine brush. All bioassays were carried out simultaneously under the same conditions of the insect rearing following a completely randomized design.

2.5. Behavioral bioassays

Two behavioral locomotory bioassays were carried out with third-instar *P. nigrispinus* and *S. cincticeps* nymphs – one using arenas fully-treated with insecticide and the other using half-treated arenas (Guedes et al., 2009; Corrêa et al., 2011). Filter papers (Whatman No. 1; 9 cm diameter) were treated with insecticide (or water) as previously described (Section 2.4). The insecticide concentrations used were the same field rates used for the time-mortality bioassays since no mortality was observed during the exposure time (10 min) in any treatment including the control. The inner walls of each Petri dish were coated with Teflon® PTFE to prevent insect escape. Arenas with individual (third-instar nymphs) *P. nigrispinus* or *S. cincticeps* were used for each insecticidal treatment in each behavioral bioassay (fully- and half-treated arenas). Twenty insects (i.e. replicates) were used for each combination of insecticide treatment and predator species (including the control) in the bioassays with fully- and half-treated arenas. In each trial, the filter paper was replaced, and the side on which the insect was released in the arena was randomly established in each trial.

The insect movement within each arena was recorded for 10 min and digitally transferred to a computer using an automated video tracking system equipped with a CCD camera (ViewPoint Life Sciences Inc., Montreal, Canada). The arena images were either undivided (for the bioassays on insecticide fully-treated arenas) or divided into two symmetrical zones (one treated and the other untreated, for the bioassays on half-treated arenas). The parameters recorded were: distance walked (cm), walking velocity (cm s^{-1}), resting time (s) and the number of stops in the arena, and proportion of time spent in each half of the arena (for the half-treated arenas). The insects spending less than 1 s on the insecticide-treated half of the arena were considered repelled, while the ones remaining less than 50% of the time on such treated half were considered irritated (Cordeiro et al., 2010).

2.6. Statistical analyses

The results of the time-mortality bioassays were subjected to Probit analysis using PROC PROBIT (SAS Institute, 2008), generating concentration-mortality curves and the selectivity and toxicity rates were calculated. To measure the selectivity of insecticides on predator species, we calculated the differential selectivity with 95% confidence intervals based on the values of LC$_{50}$ of insecticides for pest (*A. gemmatalis*) and for predators (*P. nigrispinus* and *S. cincticeps*) (Robertson and Preisler, 1992). The time-mortality data were subjected to survival analysis using the non-parametric procedure LIFETEST (SAS Institute, 2008). This procedure allows the estimate of survival curves obtained through Kaplan–Meier estimators generated from the proportion of third-instar nymphs surviving from the beginning to the end of the experiment. The overall results for locomotory bioassays were subjected to multivariate analysis of variance (PROC GLM using the MANOVA statement; SAS Institute, 2008). Each parameter was subsequently subjected to univariate analysis of variance, and Tukey's HSD test ($p < 0.05$), when appropriate (PROC UNIVARIATE, SAS Institute, 2008). Pairwise dif-

ferences in the time spent in each half of half-treated arenas (i.e., insecticide avoidance) were tested using paired Student's *t* test ($p < 0.05$) for each insecticide and species. Homogeneity of variance and normality of errors were checked and data were transformed when necessary (PROC UNIVARIATE; GPLOT PROC, SAS Institute, 2008).

3. Results

3.1. Concentration-mortality bioassays

Concentration-mortality curves for the pest *A. gemmatalis* and the predators *P. nigrispinus* and *S. cincticeps* showed low χ^2 values (<11.00) and high *p*-values (>0.09), indicating the data adequacy to the PROBIT model used to estimate the mortality curves. This allowed the estimation of the LC$_{50}$'s (Table 1).

Spinosad had the highest toxicity to *A. gemmatalis* followed by chlorantraniliprole, methamidophos and deltamethrin, with relative toxicity of 32.20, 739.43 and 1074.07, respectively (Table 1). The insecticides spinosad (LC$_{90}$ = 0.16 (0.09–0.35)), chlorantraniliprole (LC$_{90}$ = 8.90 (4.40–27.51)), deltamethrin (LC$_{90}$ = 44.40 (33.41–66.64)) and methamidophos (LC$_{90}$ = 50.86 (28.90–158.95)) are probably effective in controlling *A. gemmatalis* because the LC$_{90}$ of these insecticides in our experimental conditions were lower than the field label rate. Chlorantraniliprole was safe to *P. nigrispinus* and *S. cincticeps*, making it impossible to estimate the LC$_{50}$ for this insecticide because predators showed no mortality greater than 10% at concentrations 10 times higher than the field label rate (i.e., 133.4 µg a.i. mL^{-1}). Methamidophos and deltamethrin had the highest toxicity, respectively, in relation to spinosad for *P. nigrispinus* nymphs (Table 1). Against *S. cincticeps*, deltamethrin was the most toxic insecticide followed by methamidophos and spinosad (Table 1). Spinosad and particularly chlorantraniliprole showed higher toxicity to the pest than to the predators, unlike deltamethrin and methamidophos whose toxicity to the pest species was similar to those of both predators (Table 1).

3.2. Time-mortality bioassays

The survival analysis of predatory stinkbugs exposed to dried insecticide residues indicated significant differences among treatments for both species, *P. nigrispinus* (Log-rank test, χ^2 = 259.91, d.f. = 4, $p < 0.001$) and *S. cincticeps* (Log-rank test, χ^2 = 297.48, d.f. = 4, $p < 0.001$). The survival of *P. nigrispinus* and *S. cincticeps* nymphs was 100% in the control (without insecticide exposure) after 500 h of exposure, while the insecticides methamidophos, spinosad and deltamethrin led to 100% mortality of *P. nigrispinus* after 55, 60 and 150 h, respectively, and *S. cincticeps* after 60, 100 and 280 h, respectively (Fig. 1). Chlorantraniliprole led to 25% mortality of *P. nigrispinus* and 30% for *S. cincticeps* after 500 h exposure (Fig. 1). Such differences were reflected in the median survival time (LT$_{50}$) observed for each insecticide, with chlorantraniliprole leading to higher LT$_{50}$'s. The LT$_{50}$'s to *P. nigrispinus* were 13.52, 14.60, 24.61 and 442.61 h for the insecticides methamidophos, spinosad, deltamethrin and chlorantraniliprole, respectively, and LT$_{50}$'s to *S. cincticeps* 17.12, 17.98, 19.30 and 366.77 h for methamidophos, deltamethrin, spinosad. The median survival time was not estimated for insects without insecticide exposure because of the 0% mortality observed.

3.3. Behavioral bioassays

3.3.1. Behavioral bioassays in fully-treated arenas

The mobility parameters of *P. nigrispinus* and *S. cincticeps* in arenas fully-treated with insecticides showed significant differences

Table 1
Relative toxicity of four insecticides to third-instar velvetbean *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) and relative toxicity and selectivity (related to the velvetbean toxicity data) of four insecticides to third-instar *Podisus nigrispinus* and *Supputius cincticeps* (Heteroptera: Pentatomidae).

Insect	Insecticides	No. insects	Slope (SE)	LC$_{50}$ (95% FL) µg a.i. mL^{-1}	Relative toxicity (95% CI)	Differential selectivity (95% CI)	χ^2	p
Anticarsia gemmatalis	Spinosad	224	1.23 (0.16)	0.01 (0.01–0.02)	1.00 (0.56–1.79)	–	5.63	0.34
	Chlorantraniliprole	256	0.99 (0.13)	0.46 (0.30–0.69)	32.20 (18.24–56.84)	–	2.25	0.90
	Methamidophos	256	1.87 (0.30)	10.50 (6.68–16.32)	739.43 (436.76–1251.83)	–	10.87	0.09
	Deltamethrin	256	2.76 (0.29)	15.25 (12.72–18.73)	1074.07 (683.17–1688.62)	–	5.69	0.46
Podisus nigrispinus	Chlorantraniliprole	256	–	–	–	–	–	–
	Methamidophos	288	2.14 (0.23)	18.45 (15.04–22.80)	1.00 (0.75–1.33)	1.76 (1.19–2.59)	5.14	0.64
	Deltamethrin	160	1.83 (0.33)	36.04 (25.77–61.47)	1.95 (1.25–3.05)	2.36 (1.52–3.66)	2.82	0.42
	Spinosad	224	2.19 (0.26)	49.86 (39.29–62.28)	2.70 (2.00–3.66)	3512.54 (2199.33–5609.84)	4.73	0.45
Supputius cincticeps	Chlorantraniliprole	256	–	–	–	–	–	–
	Deltamethrin	192	1.83 (0.24)	8.36 (6.18–10.96)	1.00 (0.68–1.48)	0.55 (0.39–0.77)	3.14	0.53
	Methamidophos	256	1.74 (0.22)	19.80 (15.34–25.44)	2.37 (1.64–3.43)	1.89 (1.25–2.84)	1.47	0.96
	Spinosad	256	2.01 (0.21)	47.98 (38.18–60.29)	5.74 (4.02–8.19)	3379.88 (2116.67–5396.98)	6.09	0.41

among insecticides (df$_{num/den}$ = 16/620.81; Wilks' lambda = 0.8491; F = 2.13; p = 0.0061), predators (df$_{num/den}$ = 4/203; Wilks' lambda = 0.8928; F = 6.09; p < 0.0001) and the interaction of predators × insecticides (df$_{num/den}$ = 16/620.81; Wilks' lambda = 0.8097; F = 2.78; p = 0.0002). Univariate analyses of variance for mobility parameters varied for walked distance ($F_{(9;206)}$ = 1.93; p = 0.04),

walking velocity ($F_{(9;206)}$ = 2.70; p = 0.005), resting time ($F_{(9;206)}$ = 3.71; p = 0.0002) and number of stops ($F_{(9;206)}$ = 3.39; p = 0.0007). The locomotor activity of *P. nigrispinus* when exposed to surfaces treated with spinosad was significantly lower compared to deltamethrin (Fig. 2). As for *S. cincticeps*, the results were distinct from *P. nigrispinus* and all insecticides caused decreased locomotor activity compared to the control treatment (with water) (Fig. 2).
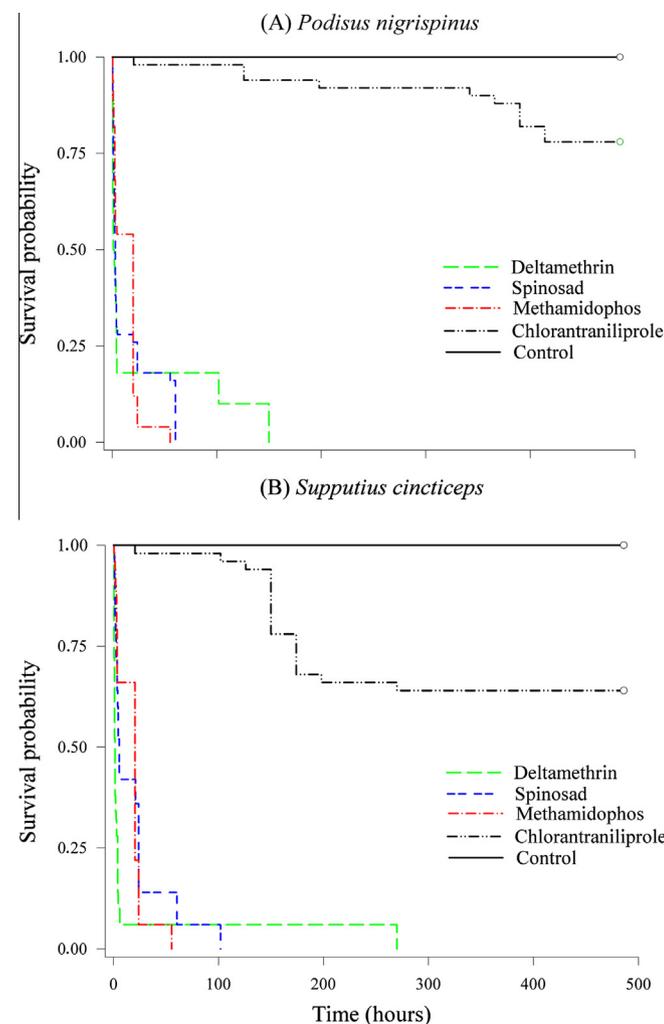
3.3.2. Behavioral bioassays in half-treated arenas

The time spent in each half of the arena half-treated with insecticides showed significant differences for *P. nigrispinus* with the insecticides methamidophos ($T_{(14)}$ = 2.42; p = 0.03) and spinosad ($T_{(14)}$ = 2.26; p = 0.04) and, for *S. cincticeps*, with methamidophos ($T_{(17)}$ = 2.52; p = 0.02), spinosad ($T_{(17)}$ = 2.13; p = 0.04) and deltamethrin ($T_{(23)}$ = 3.00; p < 0.01). The proportion of time in each half of the arena did not differ between the treated and untreated half of the arena for *P. nigrispinus* with deltamethrin and chlorantraniliprole and for *S. cincticeps* with chlorantraniliprole (p > 0.05) (Fig. 3).

Tracks representative of the typical walking behavior of third instar from both predatory stinkbugs species on arenas partially impregnated with dried insecticide residues are shown in Fig. 4. Behavioral avoidance to insecticide-treated surfaces was recognized through its two components – insecticide repellence (i.e., avoidance without contact) and insecticide irritability (i.e., avoidance after contact). Insecticide repellence was not observed in any of the insects used in this bioassay. However, insecticide irritability occurred in both predator species to the insecticides methamidophos and spinosad. In addition, *S. cincticeps* also showed irritability to deltamethrin.

4. Discussion

In this study we assessed the efficacy of residues of four neurotoxic insecticides to control the velvetbean caterpillar (*A. gemmatalis*), and subsequently evaluated the toxicity of these compounds to the predatory stinkbugs *P. nigrispinus* and *S. cincticeps* constantly reported in crops such as soybean and eucalyptus in Brazil (Matos-Neto et al., 2002; Zanuncio et al., 2004; Silva et al., 2009; Pires et al., 2011). The insecticides methamidophos (organophosphate) and deltamethrin (pyrethroids) were less toxic to *A. gemmatalis* and more toxic to predators; more recent compounds such as the bioinsecticide spinosad and, mainly chlorantraniliprole that showed the highest toxicity to this pest and lower toxicity to predators. Higher toxicity of the insecticides methamidophos and deltamethrin is mainly due to the wide action spectrum of these insecticides that, in general, have lower selectivity

Fig. 1. Survival curves of two predatory stinkbug species, *Podisus nigrispinus* (A) and *Supputius cincticeps* (B) (Heteroptera: Pentatomidae), exposed to deltamethrin, spinosad, methamidophos, chlorantraniliprole, and water (control).
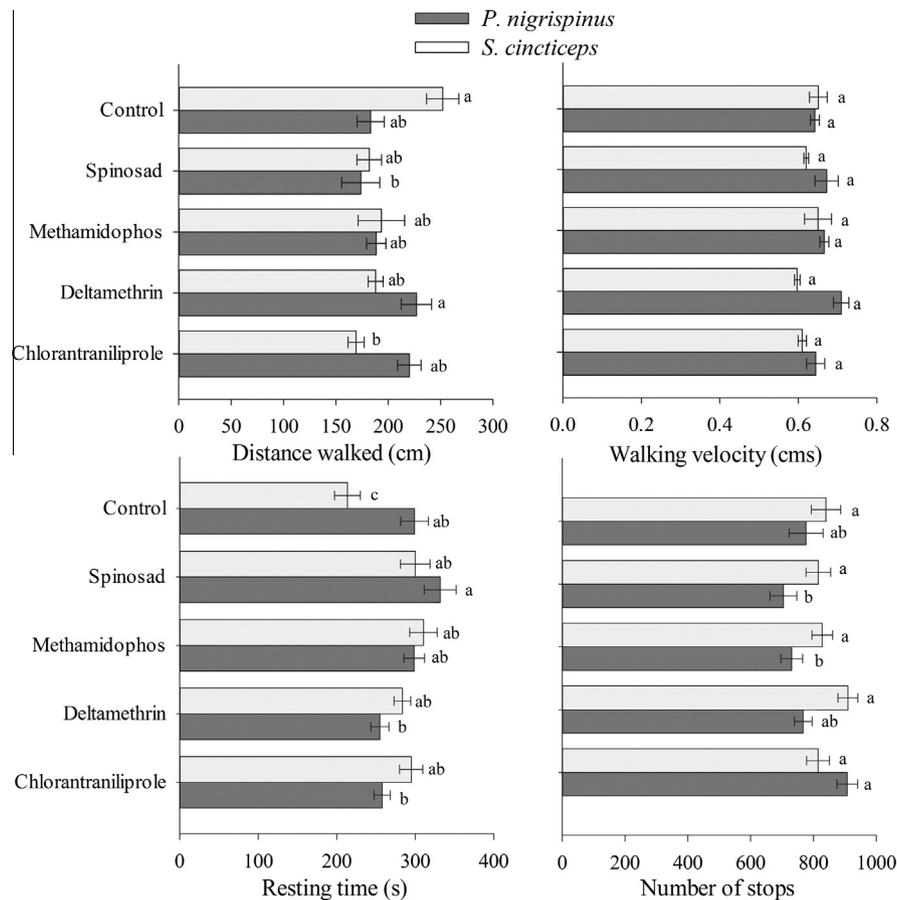
Fig. 2. Distance walked (±SEM), walking velocity (±SEM), resting time (±SEM) and number of stops (±SEM) during 10 min exposure of third-instar *Podisus nigrispinus* and *Supputius cincticeps* (Heteroptera: Pentatomidae) on filter paper arenas (9 cm diameter) fully-treated with dried insecticide residues. Bars with the same letter do not differ significantly (Tukey's HSD test at $p < 0.05$).
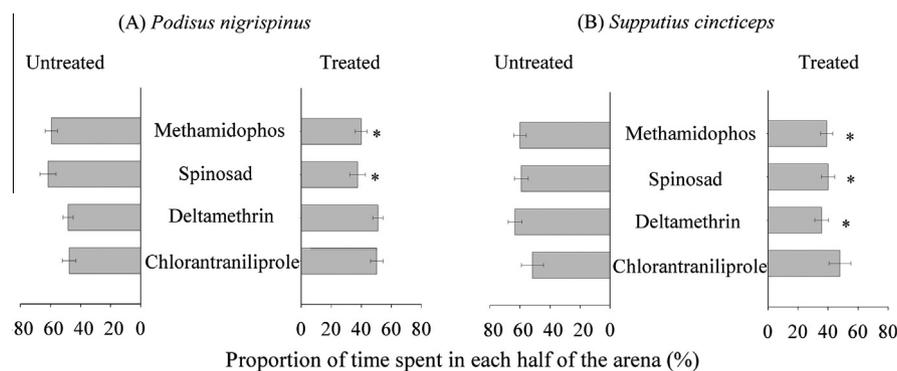

Fig. 3. Proportion of time spent by third-instar *Podisus nigrispinus* (A) and *Supputius cincticeps* (B) during 10 min exposure in each half of filter paper arenas (9 cm diameter) half-treated with dried insecticide residues. An asterisk in the bar indicates significant difference between the insecticide-treated and untreated halves of the arena (paired Student's *t* test at $p < 0.05$).

in favor of non-target species (Desneux et al., 2007; Cordeiro et al., 2010; Biondi et al., 2012a).

Spinosad showed better safety profile than deltamethrin and methamidofos, but its selectivity to non-target arthropods is disputable. Biondi et al. (2012b) reported that 71% of the reviewed studies indicated significant lethal effect of spinosad on predators (under laboratory conditions). In addition, the mortality of *P. mac-uliventris* adults increased from 20% in 24 h to 84% in 48 h and 100% in 72 h when exposed to residues of spinosad on glass surfaces (Viñuela et al., 2001), which also confirm results that pesti-

cides are more toxic on inert materials than vegetable substrates (plant) (Desneux et al., 2005; Dagli and Bashi, 2009). Plant enzymes may reduce the toxicity of the insecticide (Schuler, 1996), which can be absorbed by the waxy cuticle layer of leaves making them less available for natural enemies (Desneux et al., 2005).

The diamide chlorantraniliprole showed low toxicity to *P. nigri-spinus* and *S. cincticeps* nymphs after 500 h exposure to dried resi-dues of this insecticide and showed no mortality greater than 10% using 10x the recommended label rate after 72 h exposure. This lower toxicity for these predators was expected for chlorantranili-

Insecticides
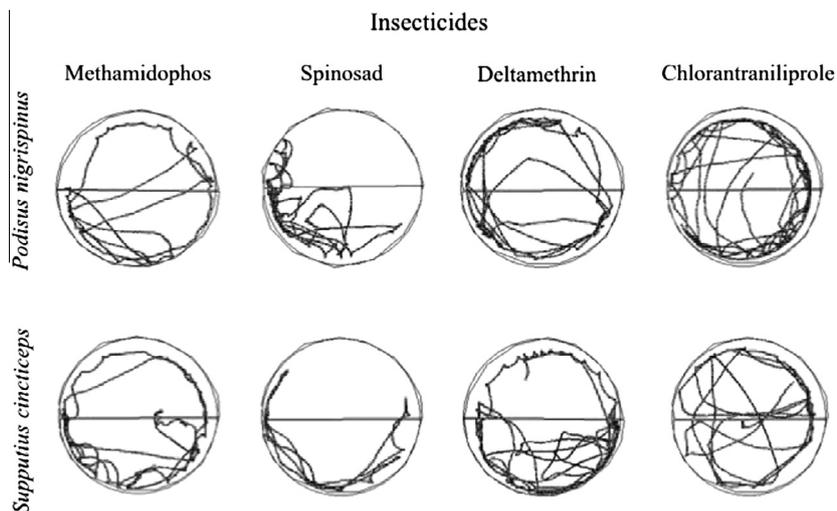
Fig. 4. Representative tracks showing the movement of individual predatory stinkbug third-instar *Podisus nigrispinus* and *Supputius cincticeps* (Heteroptera: Pentatomidae), over a 10 min period on paper-filter arenas (9 cm diameter) half-impregnated with dried insecticide residues (upper half of each arena).

prole because of its high affinity towards Lepidoptera ryanodine receptors due to the conformation and structure of the insecticide molecule (Nauen, 2006; Lahm et al., 2009). Chlorantraniliprole was also reported showing great selectivity to parasitoids, predators and mites (Dinter et al., 2008; Preetha et al., 2010; Campos et al., 2011; Biondi et al., 2012a).

Effects on behavior arising from neurotoxic compounds are not surprising and should be considered, since nerve interactions can be affected by sublethal amounts of insecticides and trigger distinct behavioral responses in comparison to individuals not exposed to insecticides (Haynes, 1988; Desneux et al., 2007; Braga et al., 2011). The insecticides used reduced the locomotor activity of *S. cincticeps* nymphs which may be an adaptive behavior that allows a lower direct exposure of predators to toxic residue (Campos et al., 2011), which did not occur in *P. nigrispinus* nymphs. Pesticides causing behavioral locomotory changes have been described in other species and can result in significant reduction in capture efficiency of the pest and its mating in areas sprayed with pesticides (Cordeiro et al., 2010; Evans et al., 2010; Griesinger et al., 2011; Biondi et al., 2012a,b; He et al., 2012).

Behavioral avoidance to insecticides is desirable in natural enemies because it reduces the exposure and increases survival in field conditions (Haynes, 1988; Desneux et al., 2007; Cordeiro et al., 2010; Campos et al., 2011). Insecticide repellence was not observed. However, predators showed significant insecticide irritability to the insecticides methamidophos, spinosad and, in the case of *S. cincticeps*, also to deltamethrin, which under field conditions can increase the survival of these predators to these insecticides because they are extremely toxic in the tested conditions in the laboratory (Cordeiro et al., 2010). However, despite of the arthropod predators avoiding insecticide contact, changes in locomotory behavior can affect the population dynamics, foraging and reproductive success of those individuals (Evans et al., 2010; Griesinger et al., 2011; He et al., 2012). Pesticides can affect the chemical communication between arthropods and reduce the ability of predators to locate their partners for mating (Griesinger et al., 2011) and consumption of pests (He et al., 2012).

In summary, we assessed the lethal and sublethal (mobility) effects of four neurotoxic insecticides used to control *A. gemmatalis* towards two pentatomid predators, *P. nigrispinus* and *S. cincticeps*. The compounds of the new generation of insecticides, especially the chlorantraniliprole, were more toxic to *A. gemmatalis* and less toxic to predators than those traditional insecticides such as orga-

nophosphates and pyrethroids. This pattern, though less obvious, was also found in behavioral walking bioassays where predators had more abrupt behavioral changes when exposed to residues of methamidophos and deltamethrin. The same pattern may also take place with other behavioral traits relevant for predator population growth and biological control (e.g., mating behavior, prey foraging, etc.), which deserves more attention. Thus, our results reinforce the need for replacement of the insecticides methamidophos and deltamethrin by more selective compounds such as chlorantraniliprole, which have lower toxicity to non-target organisms and hence allowing more sustainable IPM programs.

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