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# Demographic parameters of the insecticide-exposed predator *Podisus nigrispinus*: implications for IPM

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**Abstract** The predator *Podisus nigrispinus* (Dallas) (Heteroptera:Pentatomidae) shows potential for Integrated Pest Management programs of defoliating caterpillars in agricultural and forestry systems. Insecticides can indirectly affect caterpillar predators through consumption of treated prey. We examined the survival, reproductive and demographic parameters of the predator *P. nigrispinus* fed on caterpillars of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Erebidae) fed on soybean leaves previously exposed to four insecticides widely used in this crop, chlorantraniliprole, deltamethrin, methamidophos and spinosad. Caterpillars of *A. gemmatalis* were fed for 12 h with treated soybean leaves and offered to adults of *P. nigrispinus* over five consecutive days. Spinosad and methamidophos were proved not compatible with *P. nigrispinus* in IPM programs in the soybean agroecosystem. Deltamethrin showed low toxicity to *P. nigrispinus*. However, further data may be necessary to recommend it for IPM. Chlorantraniliprole can be considered the most promising because of low toxicity to this predator.

**Keywords** *Anticarsia gemmatalis* · Asopinae · IPM · Pentatomidae · Risk assessment

## Introduction

Generalist predators are known worldwide for their ability to control insect pests in many cultivated crops (Symondson et al. 2002). For example, most Asopinae (Heteroptera: Pentatomidae) are predatory stinkbugs

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which play a key role in the management of various pests such as lepidopteran larvae (Ribeiro et al. 2010; Zanuncio et al. 2008) in greenhouses and fields, and herbivorous Pentatomid species (De Clercq et al. 2002). These predators are able to build up their populations before pests arrive using host plants (Coll and Guershon 2002) and alternative prey as food sources (Zanuncio et al. 2005). *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) is a generalist predator native to Central and South America (Thomas 1992; Silva et al. 2009) with potential for use in integrated pest management (IPM) programs (Matos-Neto et al. 2002; Zanuncio et al. 2008).

In the soybean agro-ecosystem, and despite the potential effectiveness of biological control, many producers commonly use pesticides that could be noxious to beneficial arthropods as the main pest control method (Song and Swinton 2009). For instance, in this system pest control relies on conventional pesticides, including cyclodienes, organophosphates and pyrethroids (Baur et al. 2010). New compounds have been developed (Nauen and Bretschneider 2002) and biopesticides and biorational pesticides (relatively innocuous against non-target insects) have received considerable attention among the new ones in development and use (Rosell et al. 2008; Chandler et al. 2011). Broad-spectrum insecticides, such as pyrethroids and organophosphates, are widely used to control insect defoliators, mainly in developing countries (Sayyed et al. 2010).

An alternative to conventional pest control is IPM, which aims to maintain the pest population levels below those causing economic injury by using suitable techniques and methods in a compatible manner (van Lenteren and Woets 1988; Kogan 1998). IPM involves strategies including biological control by parasitoids, predators (Silva et al. 2009) and entomopathogens, plant resistance (Meissle et al. 2011) and pesticides when required. However, only selective insecticides to other control strategies should be used to maintain agriculture sustainability (Zalucki et al. 2009). When used as part of IPM program, biological control can enhance sustainability by reducing dependence on chemicals (Kogan 1998; Bueno et al. 2011).

The compatibility of pesticides with natural enemies is important in IPM programs (Desneux et al. 2007) since these insects are one of the key strategies of IPM and recommended as the first line of defense (Lugojja et al. 2001). To date, with reference to stinkbugs, insecticide compatibilities have been demonstrated for methoxyfenozide, pyriproxyfen and spinosad with *Picromerus bidens* L. (Heteroptera: Pentatomidae) (Mahdian et al. 2007); methoxyfenozide, tebufenozide and RH-5849 with *Orius laevigatus* (Fieber) (Heteroptera:Anthocoridae) (Amor et al. 2012); *Bacillus thuringiensis* with *Podisus maculiventris* (Say) (Heteroptera:Pentatomidae) (Mohaghegh et al. 2000); and chlorantraniliprole showed low toxicity to predators *P. nigrispinus* and *Supputius cincticeps* (Stal) (Heteroptera: Pentatomidae) during concentration-mortality and behavioral bioassays (De Castro et al. 2013). On the other hand, the pyrethroid gamma-cyhalothrin was toxic (Pereira et al. 2005) and the growth regulator diflubenzuron reduced *P. nigrispinus* fertility (Castro et al. 2012).

Tarsal contact of predators with pesticide residues on plants is the main route of exposure of these natural enemies during foraging (Mahdian et al. 2007). However, predators can also be affected by direct contact with spray droplets, ingestion of insecticides or plant sap contaminated or by feeding on contaminated prey (Mahdian et al. 2007; Cloyd and Bethke 2011). Demographic parameters may be used to evaluate sublethal effects of pesticides on the demography of both target and non-target species (Stark and Banks 2003; Stark et al. 2007; Biondi et al. 2013). Sublethal effects on population dynamics go unnoticed because they can affect the fertility of individuals (Perveen 2008) even with low mortality, as reported for *P. nigrispinus* with diflubenzuron (Castro et al. 2012). In this context, the aim of the present work was to evaluate the survival, reproductive and demographic parameters of *P. nigrispinus* fed on caterpillars of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Erebidae), a pest of soybean, exposed to some traditional insecticides (the pyrethroid deltamethrin and the organophosphate methamidophos) in addition to more recently developed compounds (the spinosyn spinosad and the diamide chlorantraniliprole).

## Materials and methods

### Insects

The predator *P. nigrispinus* and prey *A. gemmatalis* were obtained from mass-reared cultures from the Laboratory of Biological Control of Insects (LCBI) of the Institute of Applied Biotechnology in Agriculture

(BIOAGRO) at the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil. This predator is reared on pupae of the yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) under controlled environmental conditions (25 ± 2 °C, 70 ± 5 % RH, and 12:12 L:D photoperiod) (Zanuncio et al. 2005). Caterpillars of *A. gemmatalis* were reared on artificial diet (Greene et al. 1976) and their adults in wooden cages (30 × 30 × 30 cm) with screened sides, glass covers and fed cotton soaked in nutrient solution of 1 g honey, 0.1 g ascorbic acid, 0.1 g methyl para-hydroxybenzoate, and 6 g sucrose per 100 ml water (Greene et al. 1976), at the bottom of the cages.

## Insecticides

All 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, Brazil), the organophosphate methamidophos (Tamaron® BR SC; 600 g a.i. $l^{-1}$; Bayer CropScience Ltd.; Belford Roxo-RJ, Brazil), the diamide chlorantraniliprole (Premio® CS; 200 g a.i. $l^{-1}$; DuPont Brasil S.A.; Barra Mansa-RJ, Brazil) and the spinosyn spinosad (Tracer® 480 CS; 480 g a.i. $l^{-1}$; Dow AgroSciences Industrial Ltd.; São Paulo-SP, Brazil).

Pyrethroids and organophosphates act on the function of voltage-sensitive sodium channel and acetylcholinesterase, respectively (Narahashi et al. 1995; Sattelle and Yamamoto 1988). Spinosad belongs to the spynosins (nicotinic acetylcholine receptor allosteric activators). Chlorantraniliprole is an insecticide of the anthranilic diamide class with broader insecticidal activity, and it is known to control insects via activation of ryanodine receptors which leads to uncontrolled calcium release in muscle (Lahm et al. 2009).

## Reproductive and demographic parameters bioassays

Males and females of *P. nigrispinus* were isolated individually for three days after their emergence until sexually mature (Castro et al. 2012). Afterwards, 15 pairs of *P. nigrispinus* were placed individually per treatment in plastic pots (500 ml) with water provided through 2.5 ml tubes. Extra males were kept for each treatment in identical plastic pots and under the same conditions, in order to have males available to substitute those that died before their respective females.

Soybean leaves of the cultivar "BRSMT pintado" were immersed for 5 s in a solution containing one of the following 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 (1,500 µg a.i. $ml^{-1}$) and then the leaves were let to dry in shade for 1 h. Distilled water was used to prepare the insecticide solutions. Third-instar *A. gemmatalis* caterpillars were fed on the treated soybean leaves for 12 h and then presented to each couple of *P. nigrispinus* for five days following the mating period (one caterpillar per day) (Castro et al. 2012). After each day, new third-instar larvae were exposed to the treatment during those five days in order to guarantee the same age of the insects. New leaves were treated each time and new caterpillars were fed treated leaves (i.e. every day). Caterpillars that did not feed were discarded from the assays. A control was prepared by using third-instar caterpillars of *A. gemmatalis* fed on soybean leaves dipped only in distilled water. Following the five days trial, each couple of *P. nigrispinus* was fed two *T. molitor* pupae every other day until their natural death.

The egg masses were removed from the plastic pots and observations for egg hatch were made daily. The pre-oviposition, oviposition and post-oviposition periods, the number of eggs and nymphs per egg mass, the total number of eggs, nymphs and egg masses per female, egg viability, incubation period and longevity of *P. nigrispinus* female were grouped into three days age classes and used to construct demographic parameters for this predator. The reproductive parameters were subjected to the analysis of variance (ANOVA) and the means compared using Tukey's test ($P < 0.05$).

Demographic parameters were calculated according to the following formulas (Krebs 1994): (1) the net reproductive rate ($R_0$) (number of females produced per female during its life), $R_0 = \sum_{x=0}^{y} l_x m_x$; where $l_x$ is the probability of survival from birth to age $x$ per day per age class during immature and adult stages, and $m_x$ is the number of females produced per female of age $x$

and the following older class $y$; (2) generation duration ($D$) (time between the birth of the parents to that of their progeny), $D = \ln(R_o)/r_m$; (3) intrinsic rate of population increase ($r_m$) (population rate of increase per unit of time), $r_m = \ln(R_o)/D$; and (4) the time necessary for the *P. nigrispinus* population to double in size ($T$), $T = \ln(2)/r_m$. These parameters were analyzed using the SAS statistical program (SAS Institute 2000) and the Jackknife procedure (Maia et al. 2000). The demographic parameters were subjected to the ANOVA and the means compared using Tukey's test ($P < 0.05$).

## Results

Reproductive and demographic parameters of *P. nigrispinus* were not obtained for spinosad and methamidophos due to high mortality of females of this predator: 90 % after three days and 95 % after four days of feeding on caterpillars treated with these insecticides, respectively.

### Toxicity effects on life parameters

The pre-oviposition, post- and oviposition periods, incubation period, longevity, egg viability, numbers of eggs and nymphs per egg mass and number of egg masses were similar with chlorantraniliprole, deltamethrin and control (Table 1).

The numbers of eggs ($F = 5.308$, df = 2,30, $P = 0.0106$) and nymphs ($F = 5.35$, df = 2,30, $P = 0.010$) produced by female *P. nigrispinus* were higher with chlorantraniliprole and control in comparison with deltamethrin (Table 1). The number of eggs and nymphs per female per day of *P. nigrispinus* showed a peak at the beginning of the reproductive cycle of females for chlorantraniliprole, deltamethrin and control (Fig. 1a, b). Low peaks at the end of a female reproductive life cycle were also observed (Fig. 1a, b).

The survival curves of *P. nigrispinus* were similar, between the control and chlorantraniliprole (Fig. 1c), indicating that this insecticide does not directly increase or decrease longevity of this predator. Furthermore, reproductive parameters indicated no decrease in oviposition rates due to chlorantraniliprole (Table 1). The survival curve with deltamethrin was also similar to the control, but this insecticide reduced the production of eggs and nymphs of this predator (Table 1; Fig. 1).

### Toxicity effects on demographic parameters

The generation duration ($D$) was similar for chlorantraniliprole, deltamethrin and control (Table 2). However, the net reproductive rate ($R_0$) and intrinsic rate of population increase ($r_m$) were lower with deltamethrin (62.5 and 0.11, respectively) in comparison to control (115.2 and 0.13, respectively) (Table 2). The time necessary for the *P. nigrispinus* population to double

**Table 1** Life parameters (mean ± SE) of *Podisus nigrispinus* females fed on caterpillars of *Anticarsia gemmatalis* reared on soybean leaves treated with chlorantraniliprole (13.3 ppm), deltamethrin (50 ppm) and untreated leaves (control)

| Life parameters | Chlorantraniliprole | Deltamethrin | Control |
|---|---|---|---|
| Number of eggs per female | 300.18 ± 34.99 a | 177.55 ± 30.61 b | 318.18 ± 33.89 a |
| Number of nymphs per female | 274.64 ± 34.82 a | 159.64 ± 27.73 b | 290.27 ± 29.53 a |
| Pre-oviposition period (days)$^{ns}$ | 9.00 ± 0.74 | 10.73 ± 0.52 | 8.45 ± 0.87 |
| Oviposition period (days)$^{ns}$ | 24.73 ± 3.34 | 15.64 ± 3.45 | 24.00 ± 3.04 |
| Post-oviposition period (days)$^{ns}$ | 3.09 ± 0.73 | 2.09 ± 0.37 | 1.55 ± 0.28 |
| Longevity (days)$^{ns}$ | 36.82 ± 3.60 | 28.45 ± 3.80 | 34.00 ± 2.96 |
| Egg viability (%)$^{ns}$ | 90.41 ± 1.61 | 88.79 ± 2.24 | 94.45 ± 1.65 |
| Incubation period (days)$^{ns}$ | 5.01 ± 0.02 | 5.01 ± 0.01 | 5.00 ± 0.01 |
| Number of eggs per egg mass$^{ns}$ | 19.83 ± 1.76 | 17.37 ± 1.22 | 19.92 ± 1.86 |
| Number of nymphs per egg mass$^{ns}$ | 17.91 ± 1.58 | 15.70 ± 1.17 | 18.26 ± 1.74 |
| Number of egg masses$^{ns}$ | 15.91 ± 1.83 | 10.36 ± 1.85 | 17.55 ± 2.62 |

All predators in the spinosad and methamidophos treatments died before oviposition

$^{ns}$ Not significant. Means followed by the same letter within rows do not differ by ANOVA Tukey's test at 5 %

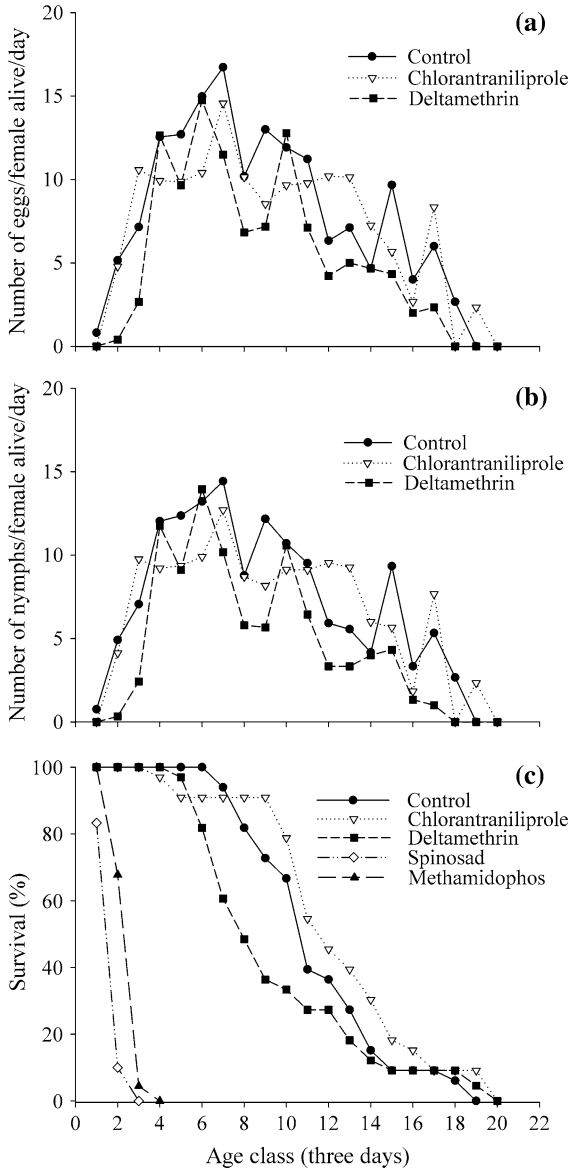


**Fig. 1** Number of eggs (**a**), nymphs (**b**) and % survival (**c**) of *Podisus nigrispinus* fed on caterpillars of *Anticarsia gemmatalis* reared on soybean leaves exposed to insecticides and untreated control

in size (*T*) was higher with deltamethrin (6.2) in comparison to chlorantraniliprole and control (5.5 and 5.3, respectively) (Table 2).

## Discussion

We assessed the survival, reproductive and demographic parameters of the predatory stinkbug *P. nigrispinus* to determine the safety of some insecticides registered for controlling *A. gemmatalis*. Conventional insecticides like the organophosphate methamidophos and the newer compound, the spinosyn spinosad were harmful to the predator *P. nigrispinus*. The pyrethroid deltamethrin affects the reproduction of this predator. However, a promising safety profile was observed for chlorantraniliprole, a novel compound available on the market.

The significantly higher number of eggs and nymphs produced per female of *P. nigrispinus* exposed to chlorantraniliprole and to untreated control compared to deltamethrin are in agreement with previous studies which reported that chlorantraniliprole was not significantly different from the control on offspring production by *O. laevigatus* (Biondi et al. 2012a) and this insecticide has showed selectivity to natural enemies (Campos et al. 2011; Preetha et al. 2009; De Castro et al. 2013). This low toxicity was expected for chlorantraniliprole, because of its high affinity for ryanodine receptors due to the structure and conformation of the insecticide molecule (Nauen 2006; Lahm et al. 2009). In contrast, pyrethroids are usually classified as very toxic to beneficial arthropods (Croft 1990; Cordeiro et al. 2010). Indeed, in previous studies deltamethrin disrupted the ability of *Anagrus nilaparvatae* (Pang et Wang) (Hymenoptera: Mymaridae) to perceive host-plant odor cues (Liu et al. 2012) and the recommended field concentration of deltamethrin for controlling *A. gemmatalis* caused 100 % mortality of *P. nigrispinus* and *S. cincticeps* nymphs after 150 and 280 h of exposure, respectively (De Castro et al. 2013). Our results suggested that the reproduction of *P. nigrispinus* could be affected by the broad-spectrum neurotoxic insecticide deltamethrin but not by the novel insecticide chlorantraniliprole, which had distinct modes of action.

The same pattern of peaks in the number of eggs and nymphs per surviving female per day for chlorantraniliprole, deltamethrin and the control in our study was observed previously in *P. nigrispinus* fed on caterpillars of *A. gemmatalis* reared on soybean leaves exposed to diflubenzuron (Castro et al. 2012) and fed on *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Vivan et al. 2002). During the adult stage, non-social insects show a pre-oviposition period, followed by reproductive stage maximum followed by a decline with insect age. In addition, ovary activation in predatory stinkbugs occurs after mating (7-d old

**Table 2** Demographic parameters (mean ± SE) of *Podisus nigrispinus* females fed on prey of caterpillars of *Anticarsia gemmatalis* reared on soybean leaves treated with chlorantraniliprole (13.3 ppm), deltamethrin (50 ppm) or untreated leaves (control)

| Treatments | $R_0$ | $D$ | $T$ | $r_m$ |
|---|---|---|---|---|
| Chlorantraniliprole | 95.94 ± 11.18 ab | 36.72 ± 1.79 a | 5.57 ± 0.20 b | 0.12 ± 0.004 ab |
| Deltamethrin | 62.50 ± 10.77 b | 37.31 ± 1.11 a | 6.23 ± 0.14 a | 0.11 ± 0.003 b |
| Control | 115.21 ± 12.27 a | 36.48 ± 1.15 a | 5.32 ± 0.20 b | 0.13 ± 0.005 a |

Means within columns followed by same letter do not differ by ANOVA Tukey's test at 5 %

$R_0$ number of females produced per female during its life, $D$ generation duration, $T$ time necessary for the *P. nigrispinus* population to double in size, $r_m$ population rate of increase per unit of time

females) with a reproductive peak in 21-d old females (Lemos et al. 2009). Sousa-Souto et al. (2006) reported that multiple matings are important for the reproductive success of *P. nigrispinus* females and the constant availability of males enables females to increase their fertility by up to 50 %. Thus, numbers of egg and nymph peaks at the end of the reproductive stage of *P. nigrispinus* could be related to the replacement of males that died before their female mates.

The survival curves of *P. nigrispinus* showed that spinosad and methamidophos caused elevated mortality of *P. nigrispinus* females, 90 % after three days and 95 % after four days of feeding on caterpillars treated with these insecticides, respectively. Spinosad has caused controversy in relation to its toxicity to natural enemies. The U.S. Environmental Protection Agency (EPA) classifies spinosad as a low risk toxicological and environmental insecticide (EPA 1997). Biondi et al. (2012b) reported that 71 and 34 % of the reviewed studies indicated significant lethal effect of spinosad on predators under laboratory and field and semi-field conditions, respectively. Spinosad caused 10 % mortality of *Geocoris punctipes* (Say) (Heteroptera: Pentatomidae) after 72 h treatment with caterpillars of *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae) fed for 6 h on treated soybean leaves (Boyd and Boethel 1998) and low mortality of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) on prey treated with this insecticide (Galvan et al. 2006). However, selectivity of spinosad on predators is under discussion because earwigs *Doru taeniatum* (Dohrn) (Dermaptera: Forficulidae) were reported to suffer 86 % mortality/intoxication 72 h after feeding on spinosad-treated *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) larvae (Cisneros et al. 2002). Furthermore, 72 h after treatment, spinosad at the maximum concentration recommended (800 mg a.i. $l^{-1}$) reduced the number of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) adults by 39.8 and 87.2 % in topical and ingestion treatments (Medina et al. 2003). Finally, significant mortality of *P. maculiventris* nymphs was found when submitted to ingestion and topical treatments of spinosad from 15 to 50 mg a.i. $l^{-1}$ onwards, respectively (Viñuela et al. 1998). It is evident that the safety profile of spinosad is unclear, although some differences might be explained because results in the laboratory can be different from those obtained in the field (Biondi et al. 2012b). Organophosphates are toxic to insects because of their ability to inactivate acetylcholinesterase (Fukuto 1990). The high mortality of *P. nigrispinus* with methamidophos is mainly due to the broad action spectrum of this insecticide rendering it as not compatible with natural enemies (Bacci et al. 2007; Preetha et al. 2009; Wang et al. 2012). Therefore, organophosphates should be replaced with relatively safe plant-protection products in IPM programs.

The survival and fertility rates observed show no impact of chlorantraniliprole, but the demographic parameters showed reduction in the reproductive capacity of *P. nigrispinus* with deltamethrin. The sublethal effects of the insecticides chlorantraniliprole and deltamethrin on *P. nigrispinus* can be explained by using demographic parameters that show how its population dynamics may be affected (Castro et al. 2012). The reduced fertility shown by *P. nigrispinus* exposed to deltamethrin resulted from a reduction in the number of eggs and nymphs per female and other demographic parameters such as $R_0$ and $r_m$. The positive values of $R_0$ (>1.0) and $r_m$ with chlorantraniliprole and deltamethrin indicate a potential for population increase of this predator with these insecticides (Medeiros et al. 2000, 2003; Castro et al. 2012). However, the lower net reproductive rate ($R_0$) and intrinsic rate of population increase ($r_m$) of *P.*

*nigrispinus* fed on caterpillars exposed to deltamethrin demonstrate a serious effect of this insecticide on the capacity for population increase of this natural enemy, similar to that found for *P. nigrispinus* fed on caterpillars exposed to diflubenzuron (Castro et al. 2012). Thus, deltamethrin adversely affects the reproduction of this predator and its use in IPM programs should be studied further.

The present study demonstrated that lethal and sublethal effects of traditional pesticides and newer compounds on the generalist predator *P. nigrispinus* via treated prey varied widely, confirming the importance of pesticide risk assessments on natural enemy population dynamic related traits (Biondi et al. 2013). *Podisus nigrispinus* was very susceptible to spinosad and methamidophos, notably because of high adult mortality which makes these insecticides observed incompatible with the tested predator in IPM programs. Although this study showed low toxicity of deltamethrin on *P. nigrispinus*, the obtained results are not sufficient to recommend it for IPM programs. Finally, chlorantraniliprole proved to be harmless, with mortality and reproductive capacity levels similar to those recorded in the untreated control group. However, a single route of pesticide exposure—through treated prey—does not represent a worst-case scenario. Consequently, specific risk assessment as well as field studies for more comprehensive assessment of the safety of these compounds to predatory stinkbugs should be undergone before implementing any IPM program.

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