



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.

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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 gemmatilis*) 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. gemmatilis*. The recommended field concentration of deltamethrin, methamidophos and spinosad for controlling *A. gemmatilis* 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. gemmatilis* in soybeans.

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1. Introduction

Insecticide selectivity and impact on natural enemies are key components of Integrated Pest Management (IPM) programs (Met-

calf, 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

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(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 gemmatilis Hübner (Lepidoptera: Noctuidae) is one of the major lepidopteran pests of soybeans occurring 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. gemmatilis*. We assessed the acute toxicity and behavioral sublethal response of the predators *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stål) (Heteroptera: Pentatomidae) exposed to deltamethrin, methamidophos, spinosad and chlorantraniliprole. These insecticides are used for *A. gemmatilis* control and this study may help optimizing combined use of pesticides and natural enemies for management of *A. gemmatilis*, 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. gemmatilis* 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, $70 \pm 5\%$ 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. gemmatilis* 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. gemmatilis* 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. gemmatilis* 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. gemmatilis* 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. gemmatilis* (chlorantraniliprole-13.3 μ g a.i. mL⁻¹, deltamethrin-50 μ g a.i. mL⁻¹, spinosad-240 μ g a.i. mL⁻¹ and methamidophos-1500 μ g 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)

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