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Research Article

Spodoptera frugiperda (Noctuidae) fed on transgenic maize can transfer Bt proteins to *Podisus nigrispinus* (Pentatomidae)

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ABSTRACT: An important concern with the use of genetically modified (GM) plants expressing *Bacillus thuringiensis* (Bt) insecticidal toxins is the deleterious effect on non–target organisms. The predatory stink bug *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) is used in biological control programs and may be exposed to Bt toxins. This study evaluated the indirect effects of different Cry proteins on *P. nigrispinus* with the prey *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), fed on simple or pyramided Bt maize genotypes. The experiment was carried out in a completely randomized design with three treatments: i) Isohybrid (not Bt), ii) Herculex® (transgenic maize encoding Cry1F protein) and iii) PowerCore® (pyramidal transgenic maize encoding the Cry1F, Cry1A.105, and Cry2Ab2 proteins), which were used to feed *S. frugiperda* for 48 h. The caterpillars were used as prey by *P. nigrispinus* females. We evaluated the presence of Cry proteins, consumed prey biomass (predation), oviposition period, number of postures, number of eggs, number of eggs per posture, number of nymphs, egg viability, embryonic period, female longevity and development, and survival rates of immature. The results show that different Cry proteins move through the food chain of *P. nigrispinus* and provide evidence that the ingestion of three different proteins does not lead to unexpected synergistic effects. However, Cry toxins promoted histopathological changes in midgut cells of *P. nigrispinus*.

Keywords: *Bacillus thuringiensis*, biological control, non–target effect, predator

Introduction

Genetically modified (GM) crops that contain genes introduced by genetic engineering to control insects express pesticidal proteins (Cry) from the bacterium *Bacillus thuringiensis* (Bt) that may have insecticidal action on insects of the orders Lepidoptera, Coleoptera, Hymenoptera, and Diptera (Abbas, 2018). Because of their high control efficacy and ease use, Bt crops have increased rapidly (Huang, 2020).

Nonetheless, Cry proteins can be transferred directly or indirectly to other non–target arthropods insensitive to Cry toxins or natural enemies, particularly predators and parasitoids (Dutra et al., 2012). Knowledge of the possible effects of Cry toxins on non–target organisms supports the risk assessment that precedes the commercial cultivation of any GM plant, especially because these toxins move through different trophic levels (Meissle and Romeis, 2018).

In modern crops, several Cry proteins and others have been stacked or pyramided, either by simultaneous multiple gene transfer or by conventional breeding methods of plants containing individual transgenes (Svobodová et al., 2017b). Different Cry proteins in stacked or pyramidal Bt plants may interact synergistically and lead to unexpected effects on non–target species (Hilbeck and Otto, 2015).

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), is an agricultural pest original from tropical and subtropical regions and causes significant losses in various crops (e.g. maize, cotton, and soybean) (Tian et al., 2014). The widespread use of Bt results in the rapid occurrence of resistance of *S. frugiperda* to Cry1F maize (TC 1507) affecting field control of this pest in Argentina, Brazil, Puerto Rico, and the United States (Huang, 2020).

Podisus nigrispinus (Dallas, 1851) (Hemiptera: Pentatomidae) naturally occurs in a variety of agro–ecosystems and is the most common species of Asopinae in Brazil, also used for biological control in agricultural and forestry systems (Zanuncio et al., 2016). In addition to feeding on prey, *P. nigrispinus* has the habit of sucking parts of plants to obtain water and minerals (Torres et al., 2010; Vieira et al., 2018), such behavior increases the risk of contact with Cry toxins when exposed to Bt plants. For these predatory insects, exposure to Cry proteins can cause physiological damage, affecting behavior, development, and longevity (Cunha et al., 2013; Jesus et al., 2014; Carvalho et al., 2018; Marques et al., 2018).

This study evaluated the flow of different Cry proteins in *P. nigrispinus* fed on *S. frugiperda* previously fed on simple or pyramided Bt maize genotypes, as well as the developmental, predatory, and reproductive ability of the predator. In addition, we assessed toxicity and histopathological changes mediated by these Cry proteins in the midgut of *P. nigrispinus*.

Materials and Methods

Plants

The hybrids, Isohybrid (Dow AgroSciences, São Paulo, SP, Brazil), non–Bt isogenic maize of the same genetic background used as control, Herculex® (TC1507, Dow AgroSciences LLC, Indianapolis, IN, USA), transgenic maize coding for protein Cry1F and, PowerCore® (MON 89034 × TC1507 × NK603, Monsanto Technology LLC and Dow AgroSciences LLC, Indianapolis, IN, USA), transgenic pyramidal maize coding for proteins Cry1F, Cry1A.105, and Cry2Ab2, were used in this experiment. Maize was kept in a greenhouse in 8 L pots. The cultivation was carried out according to the recommendations for Brazil, without the application of insecticides, fungicides and, herbicides.

Insects

Eggs of *Spodoptera frugiperda* were obtained from mass–rearing conditions in Diamantina, Minas Gerais State, Brazil. The insects were kept at 25 ± 2 °C, 75 ± 5 % RH and a 12:12 (light: dark) photoperiod. After hatching, caterpillars were fed on artificial diet (Greene et al., 1976).

Adults of *Podisus nigrispinus* were obtained from mass–rearing kept at 25 ± 2 °C, 75 ± 5 % RH and a 12:12 (light: dark) photoperiod. These insects were fed on *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae) pupae and *Eucalyptus grandis* (Myrtaceae) leaves *ad libitum* (Neves et al., 2010). Water was supplied in an anesthesia tube type fixed at the cover of the plastic pot (500 mL) and with its extremity closed with cotton.

Bioassays

Bioassays were carried out in laboratory at 25 ± 2 °C, 75 ± 5 % RH and a 12:12 (light: dark) photoperiod. The experiment was carried out in a completely randomized design with three maize genotypes: i) Isohybrid, ii) Herculex® and iii) PowerCore®. We used 14 replications, each replicate included a *P. nigrispinus* female, fed on *S. frugiperda*, that consumed Bt or non–Bt maize leaves.

Caterpillars of *S. frugiperda*, 10 days old, were individualized in transparent plastic pots (500 mL) and fed *ad libitum* with Isohybrid, Herculex®, or PowerCore® maize leaves at the vegetative stage V3 for 48 h (the leaves were renewed every 24 h). The use of caterpillars at 10 days of age was determined by preliminary tests to simulate certain resistance to Cry proteins. The caterpillars were weighed before and after feeding on each evaluated maize genotype.

Females of *Podisus nigrispinus* (3 days old) were individualized and fed daily for 3 d with one *S. frugiperda* fed with each maize genotype. The amount of food supplied to the female of *P. nigrispinus* was sufficient and determined by preliminary tests, where the prey was not fully consumed by the predator during the 24 h. The biomass consumed (predation) by *P. nigrispinus* was determined daily. For this, *S. frugiperda* was weighed before and after 24 h of contact with the predator. Caterpillars with tegument lesions, lack of mobility, or partially or fully sucked body contents were considered predated.

Females *Podisus nigrispinus* were individualized after predation of *S. frugiperda* with a male in transparent plastic pots (500 mL) and fed on *T. molitor* larvae and pupae. From these adults, the following reproduction data were obtained: oviposition period, number of oviposition events, number of eggs, number of eggs per oviposition, number of nymphs, egg viability, embryonic period, and longevity of females. Eggs were collected daily and placed in Petri dishes (9 cm diameter) to determine incubation period and viability. Each Petri dish contained a moistened cotton ball attached to the lid to prevent dehydration of eggs.

Ten nymphs per treatment (from different egg masses and emerged on the same day), after reaching the second instar, were collected and individualized in transparent plastic containers. Nymph development was observed until adult emergence to evaluate the duration of nymphal instars.

Data on biomass gain, biomass consumed, reproduction, and development were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey test at 5 % significance level with the statistical program SISVAR. For ANOVA, original data on the percentage of egg viability, number of eggs, and nymphs were transformed using arcsine ($\chi^{0.5}$) to meet the assumptions of normality.

Cry Detection

To evaluate the presence of Cry1F protein and Cry1A.105 and Cry2Ab2 proteins in maize plants, *S. frugiperda* and, *P. nigrispinus*, we used the Envirologix QuickStix™ kit for Cry1F and Cry2A. Three samples with leaf tissue, *S. frugiperda* or *P. nigrispinus* were used for the detection of Cry proteins in Herculex® and PowerCore® genotypes and four samples for Isohybrid (two Envirologix QuickStix™ strips for Cry1F and two for Cry1A.105 and Cry2Ab2 were used in each sample).

In each Eppendorf tube containing the samples, 0.5 mL of the extraction buffer was added then the samples were macerated with the disposable pestle. The insects were macerated completely (one *S. frugiperda* or *P. nigrispinus*) and one disc (1 cm in diameter) of maize tissue was ground by rotating the pestle against the sides of the tube with twisting motions. The Eppendorfs were closed and shaken carefully for 30 sec. Subsequently, the tubes were placed in a holder and an Envirologix QuickStix™ Cry1F strip for Herculex® maize samples and an Envirologix QuickStix™ Cry2A strip for PowerCore® maize were added. The strips were kept inside each sample for 10 min to interpret the results.

In samples with the presence of Cry1F or Cry1A.105 and Cry2Ab2, a second line (test line) was detected in the region between the control line and the lower end of the strip. For negative samples, the strip only developed the control line.

Histological Analysis

Nine *P. nigrispinus* females per treatment, after predation of *S. frugiperda*, were cryoanesthetized at -4 °C and dissected under a stereomicroscope for midgut removal, which was subsequently transferred to aqueous 2.5 % glutaraldehyde for 24 h. The samples were dehydrated in a graded ethanol series (70 %, 80 %, 90 %, and 95 %) for 10 min and embedded in Historesin (Leica Biosystems Nussloch GmbH, Wetzlar, Germany) for 24 h. Slices 3 µm thick were obtained on microtome, stained with hematoxylin and eosin, and analyzed under a light microscope. The images (photomicrographs) were submitted to Adobe Photoshop software for contrast adjustment, white balance, balance, and scaling. Subsequently, the boards were made from photomicrographs using Corel DRAW 12 (Corel Incorporated) software.

Results

The QuickStix™ immunochromatographic strip test for Cry1F and Cry2A was negative for maize leaf, *S. frugiperda*, and *P. nigrispinus* samples in the Isohybrid treatment. The development of the control line indicates that the strip worked correctly. The results of QuickStix™ strips with Herculex® and PowerCore® were positive for maize leaves, *S. frugiperda* fed on Bt maize, and samples with the predator *P. nigrispinus* that predated *S. frugiperda*.

Biomass gain of *S. frugiperda* without exposure to Cry protein (Isohybrid) is approximately nine–fold higher than in caterpillars fed on Herculex® or PowerCore® maize leaves for 48 h (Figure 1).

The consumed biomass of *S. frugiperda* by *P. nigrispinus* was higher in Isohybrid treatment (0.061 ± 0.03 mg) whereas Herculex® (0.031 ± 0.01 mg) and PowerCore® (0.039 ± 0.01 mg) did not differ by the Tukey test at 5 % significance (Figure 2).

The results for reproduction parameters were similar between the treatments with the oviposition period (17.16 ± 8.60 to 24.33 ± 9.83 days), number of oviposition events (15.25 ± 6.45 to 21.41 ± 8.36), number of eggs (219.66 ± 153.28 to 339.75 ± 195.83), number of eggs per oviposition (12.28 ± 3.90 to 14.67 ± 4.78), number of nymphs (187.50 ± 144.21 to 289.58 ± 200.30), egg viability (76.05 ± 27.94 to 84.15 ± 7.78 %), embryonic period (4.29 ± 1.36 to 4.77 ± 0.12), and longevity of females (25.33 ± 9.51 to 33.08 ± 10.84 days) (Table 1).

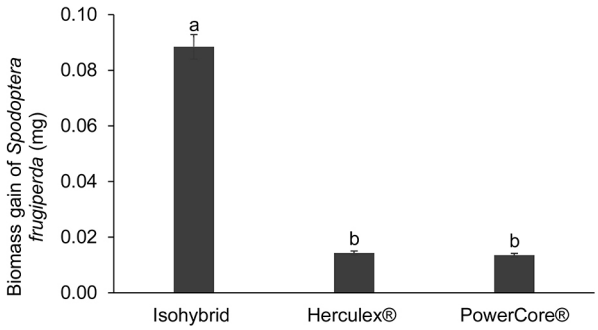

Figure 1 – Biomass gain of *Spodoptera frugiperda* (mg) after 48 hours feeding on Isohybrid, Herculex® or PowerCore® maize genotypes. Means followed by the same letter do not differ from each other at 5 % significance by the Tukey test.

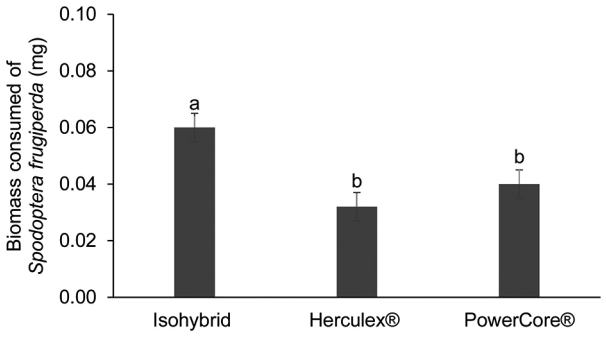

Figure 2 – Biomass consumed of *Spodoptera frugiperda* (mg), after 48 hours feeding on Isohybrid, Herculex® or PowerCore® maize genotypes, by the predator *Podisus nigrispinus*. Means followed by the same letter do not differ from each other at 5 % significance by the Tukey test.

Table 1 – Reproduction parameters (mean ± standard error) of *Podisus nigrispinus* (Hemiptera: Pentatomidae) after predation of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed Isohybrid, Herculex® or PowerCore® maize genotypes. Diamantina, Minas Gerais, Brazil.

Parameters	Isohybrid	Herculex®	PowerCore®
Oviposition (days)	18.41 ± 7.49 a	24.33 ± 9.83 a	17.16 ± 8.60 a
Number of postures	17.25 ± 8.40 a	21.41 ± 8.36 a	15.25 ± 6.45 a
N. of eggs	222.00 ± 126.34 a	339.75 ± 195.83 a	219.66 ± 153.28 a
N. of eggs per posture	12.28 ± 3.90 a	14.67 ± 4.78 a	13.84 ± 4.41 a
N. of nymphs	187.91 ± 114.74 a	289.58 ± 200.30 a	187.50 ± 144.21 a
Egg viability (%)	81.73 ± 10.00 a	76.05 ± 27.94 a	84.15 ± 7.78 a
Embryo period (days)	4.77 ± 0.12 a	4.29 ± 1.36 a	4.74 ± 0.13 a
Female longevity (days)	27.91 ± 9.57 a	33.08 ± 10.84 a	25.33 ± 9.51 a

Means followed by the same letter in the line do not differ between them at 5 % of significance by the Tukey test.

There were no differences in the duration of the nymphal period of *P. nigrispinus*, with values ranging from 2.38 ± 0.18 to 2.70 ± 0.15, 3.40 ± 0.16 to 3.86 ± 0.26, 2.50 ± 0.22 to 2.88 ± 0.35 and, 3.29 ± 0.18 to 4.00 ± 0.38 days, for I, II, III and IV instar respectively. However, the 5th instar nymph was 2 d longer when predators fed on *S. frugiperda* from Isohybrid genotype and 5 d for Herculex® and PowerCore® (Table 2).

The midgut of *P. nigrispinus* females not exposed to Cry proteins had the epithelium with a single layer of columnar cells with a well-developed apical portion brush border and the cytoplasm with some vacuoles and secretory vesicles (Figures 3A, 3B and 3C).

Histopathological changes were found in the midgut of *P. nigrispinus* after exposure to Cry1F, Cry1A.105 and Cry2Ab2 proteins. The epithelium was irregular with signs of degeneration characterized by the release of cell debris containing pyknotic nucleus into the gut lumen (Figures 3D, 3E, 3G, and 3H).

After exposure to Cry proteins, the midgut epithelium showed apical cell protrusions, increase in the number of secretory vesicles and vacuoles of varying sizes in the epithelial cytoplasm, located in the cellular apical region, where the cytoplasmic content protrudes into the midgut lumen (Figures 3E, 3F, 3G and 3I).

Discussion

In Brazil, Bt-resistant populations of *S. frugiperda* has been selected mainly due to the rapid dispersion of Bt maize plantations, poor implementation, and adoption of resistance management practices, and low refuge compliance (Farias et al., 2014; Moscardini et al., 2020). Thus, implementing effective insect resistance management (IRM) strategies is essential to ensure the success of the Bt crop technology (Yang et al., 2017).

Table 2 – Duration in days (mean ± standard error) of *Podisus nigrispinus* (Hemiptera: Pentatomidae) instars after predation on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed with Isohybrid, Herculex® or PowerCore® maize genotypes. Diamantina, Minas Gerais, Brazil.

	Duration (days)		
Instar	Isohybrid	Herculex®	PowerCore®
I	2.50 ± 0.17 a	2.70 ± 0.15 a	2.38 ± 0.18 a
II	3.40 ± 0.16 a	3.86 ± 0.26 a	3.63 ± 0.18 a
III	2.50 ± 0.22 a	2.86 ± 0.14 a	2.88 ± 0.35 a
IV	3.50 ± 0.22 a	4.00 ± 0.38 a	3.29 ± 0.18 a
V	7.33 ± 0.67 a	5.14 ± 0.14 b	5.50 ± 0.22 b
Nymphal period	19.11 ± 1.11 a	18.43 ± 0.75 a	17.16 ± 0.48 a

Means followed by the same letter in the line do not differ between them at 5 % of significance by the Tukey test.

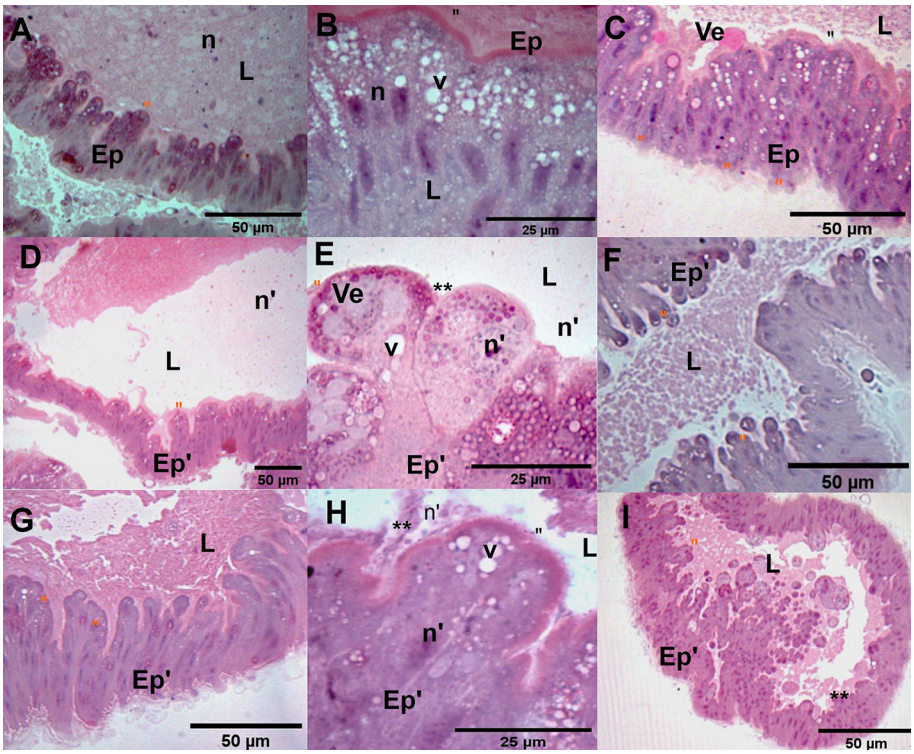

Figure 3 – Histological sections of the midgut of *Podisus nigrispinus* (Hemiptera: Pentatomidae) after predating *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed on Isohybrid (A, B and C), Herculex® (D, E and F) or PowerCore® (G, H and I) maize. Anterior (A, D and G), middle (B, E and H) and posterior (C, F, and I) midgut regions showing effects of proteins Cry1F, Cry1A.105, and Cry2Ab2 with high number of vacuoles (v), vesicles (Ve), nuclei (n) with condensed chromatin (n'), cellular fragments (**) released into the lumen (L) and epithelium (Ep) with irregular aspect (Ep') (D, E, F, G, H and I), with columnar cells (orange ") and striated border (black ").

Therefore, target insect resistance may be delayed with the use of biological control (Pereira, 2014), including the predator *P. nigrispinus*.

The negative result of the immunochromatographic test in the Isohybrid treatment confirms that this genotype does not express Bt proteins. Positive results in Herculex® and PowerCore® treatments in leaf samples, *S. frugiperda* and *P. nigrispinus* demonstrate the transfer of different plant Cry proteins to herbivores and predators. Caterpillars that fed on maize leaves containing Cry1F or Cry1A.105 and Cry2Ab2 proteins, even for 48 h, accumulated the proteins and transferred them to *P. nigrispinus* predator. The flow of Cry proteins in the trophic level has been reported in many insects (Dutra et al., 2012; Yu et al., 2014; Marques et al., 2018; Souza et al., 2018). The amount of Bt protein ingested may differ according to the herbivorous species in terms of time and place of toxin expression in the plant, the herbivorous food ecology, as well as the amount of plant material ingested (Devos et al., 2012). However, Cry proteins are diluted from the lowest to the highest trophic levels due to excretion and digestion (Svobodová et al., 2017b; Li et al., 2017).

Predators are exposed to Bt protein concentrations ranging from 5 % to 50 % of average leaf concentrations when feeding on herbivores. Predators *Adalia bipunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae), and *Harmonia axyridis* (Palias, 1733) (Coleoptera: Coccinellidae) obtain 1 % to 30 % of the Bt protein from the prey *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae), demonstrating that these proteins are diluted at higher trophic levels (Meissle and Romeis, 2018). *Podisus nigrispinus* is a zoophytophgous predator because it also feeds on plant sap to obtain water and additional nutrients (Peluzio et al., 2018; Vieira et al., 2018) thus acquiring a higher concentrations of Bt proteins than zoophagous predators.

The physiological mechanism of Bt protein sequestration is poorly known. However, after the protein is ingested, it crosses the peritrophic matrix bind to the ABC-membrane transporter of midgut cells disrupting the epithelium (Mitsuhashi and Miyamoto, 2020). Therefore, some Cry toxins may cross the midgut barrier to be stored in the fat body (Telfer and Kunkel, 1991; Locke and Collins, 1968). Protein uptake from the hemolymph to the fat body seems to occur by endocytosis (Locke and Collins, 1966; Tojo et al., 1978). This may occur in *S. frugiperda* allowing the movement of different Cry proteins of the plant to higher trophic levels.

The higher biomass gains of *S. frugiperda* fed on Isohybrid maize than on transgenic genotypes suggest that Bt toxins may decrease consumption of plant tissues due to gut paralysis (Sikorowski and Lawrence, 1997; Prütz and Dettner, 2004). Low weight has been reported in herbivores due to slow feed rate in Bt plants (De Sousa Ramalho et al., 2011) as well as low digestion rate (Razze et al., 2011). *Spodoptera frugiperda* fed on Cry1Ab maize compared to those fed on the same non-Bt hybrid had a 20-fold reduction in their biomass (Mendes et al., 2011). The amount of food consumed by *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae), *S. frugiperda* and *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) in non-Bt cotton cultivar (DPL50) was higher than in Bt cultivars. Moreover, plants expressing two Bt toxins have higher sublethal effects on caterpillar than single toxin-expressing cultivar (Stewart et al., 2001). In this study, no statistical differences were observed between genotypes with more or less Bt proteins.

The lower predation of *P. nigrispinus* in prey fed on Bt maize may be due to the low caterpillar biomass and nutritional deficiency. Low biomass accumulation may be an indicator of lower nutritional quality (Pereira, 2014). Cotton expressing Cry1Ac (cultivar DP 404 BG Bollgard; Monsanto, Saint Louis, MO, USA) negatively affects *S. frugiperda* nutritional indexes, such as relative growth rate, relative consumption rate, relative metabolic rate, approximate digestibility, metabolic cost, ingested feed conversion efficiency, and digested feed conversion efficiency (De Sousa Ramalho et al., 2011). Lower consumption of genetically modified maize genotypes can also affect the amount of N acquired by insects. The need for N from the plant for phytophagous insects is evident, due to the central role that N plays in metabolic processes, cell structure, and genetic code (Pizzamiglio, 1991). *Dicyphus hesperus* (Knight, 1943) (Hemiptera: Miridae) feeds more on prey with high N concentrations than those with low concentrations (Vankosky and Vanlaerhoven, 2015). Thus, nutritional deficiency, such as low N in Herculex® or PowerCore® maize-fed prey, may discourage *P. nigrispinus* predation.

Spodoptera frugiperda without exposure to Cry protein (Isohybrid) had higher biomass for predation, which was quickly consumed, unlike caterpillars exposed to the protein. This study did not evaluate the number of preys consumed; however, this number is possibly higher in prey in contact with transgenic crops due to reduced nutritional value. Predation of *P. nigrispinus* nymphs and adults was higher in *S. frugiperda* fed on leaves of the transgenic cotton NuOpal® (Jesus et al., 2014). The predator *H. axyridis* has preference for older Bt-fed *S. frugiperda*, when weight differences between caterpillars exposed or not to Bt increased (Svobodová et al., 2017a). *Harmonia axyridis* likely compensated the reduction in Bt-fed *S. frugiperda* biomass by increasing the number of caterpillars consumed (Lawo et al., 2010). This partly explains the indirect effects found in this work of lower biomass consumption in *S. frugiperda* fed Herculex® or PowerCore® maize when the same number of preys was offered to predators.

Reproduction parameters of *P. nigrispinus* followed 3-day feeding on Bt-fed prey was not significantly affected. *Podisus nigrispinus* exposed to Cry proteins through different food sources have similar number of eggs per female, number of eggs per day, fecundity,

and fertility (Carvalho et al., 2012), nymph survival, duration of pre-oviposition, oviposition, and adult longevity (Santana et al., 2017). On the other hand, the oviposition period and the number of eggs per postures of *P. nigrispinus* change when predating *T. molitor* larvae fed on wheat bran containing different *B. thuringiensis* (Agree®) concentrations (Carvalho et al., 2018). Despite the differences found in oviposition periods and number of eggs, these values were higher than those found when the predator was fed on *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) (Vacari et al., 2007) and with the alternative prey *T. molitor* (Zanuncio et al., 1996; Bortoli et al., 2011), respectively.

The prolongation of the 5th instar nymph of *P. nigrispinus* fed on prey from Isohybrid genotype may be related to several factors, such as food quality (Zanuncio et al., 2001; Oliveira et al., 2011); however, a longer duration of final instars is common for these natural enemies (Zanuncio et al., 2001; Oliveira et al., 2004). Cry1A, Cry2Ab, and Cry1F proteins do not affect larval survival and developmental time of *Chrysoperla rufilabris* (Burmeister, 1839) (Neuroptera: Chrysopidae) (Tian et al., 2013). On the other hand, the duration of the 3rd instar of *P. nigrispinus* ranged from 4.90 to 3.23 days when the prey was fed on NuOpal® and non-transgenic DeltaOpal® cotton, respectively (Jesus et al., 2014). The nymphal development of this predator was four days longer when they fed on *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) treated with Bt HD-1 bioinsecticide, probably due to the poor nutritional quality of prey. The nymphal period was 21.1; 18.6 and 17.1 days for HD-1, Agree® and control, respectively (Magalhães et al., 2015).

Although Cry1F, Cry1A.105, and Cry2Ab2 have non-lethal effects for *P. nigrispinus* after predation of *S. frugiperda* fed on Bt maize genotypes, some histopathological effects occur in the digestive tract of this predator. Oliveira et al. (2016) suggest that *P. nigrispinus* digestive enzymes do not fully degrade the Cry1Ac toxin. Insect susceptibility to Bt protein varies with time and site of toxin expression in the plant, herbivorous food ecology, amount of plant material ingested (Devos et al., 2012), and the presence of specific receptors on microvilli of midgut cells (Bravo et al., 2007; Castro et al., 2017; Javed et al., 2019).

Typical features of cellular degeneration, such as irregularly shaped epithelium, nuclear chromatin condensation, apical cell protrusion, and release of cell fragments into the midgut lumen of *P. nigrispinus* show possible side effects of Bt toxins. These morphological changes may indicate a decrease in the digestive capacity of insects, as reported in *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) and *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Noctuidae) in response to the toxic effect of Bt (Barbeta et al., 2008; Castro et al., 2019).

Cell fragments released into the midgut lumen of *P. nigrispinus* after predation of *S. frugiperda* fed on transgenic maize implies cytotoxic effect causing apoptosis, a morphological pattern of programmed cell death (Ihara et al., 1998). Discarding cells by cell death (Santos et al., 2015) might be a response to damages to midgut epithelial cells after Bt ingestion. Infections, mutations, presence of viruses, toxic aggressions, such as insecticides or herbicides, thermal shock, ionizing radiation, among others are stimuli described as triggers of midgut cell death (Gonçalves et al., 2013; Daniel et al., 2014; Gonçalves et al., 2016). Despite the importance of apoptosis to eliminate damaged or infected cells that may affect their normal function, excessive apoptosis can impair tissue function (Portt et al., 2011; Clapp et al., 2012), affecting important digestion processes and consequently predation and survival.

Daily dietary intake of approximately 23 $\pm$ 0.70 ng g^{-1} Cry1Ac by wet weight of leaves and expressed by Bt cotton induces small ultrastructural changes in predator *P. nigrispinus* granulocytes and plasmatocytes (Cunha et al., 2013). Exposure to Bt proteins also causes microvilli elongation, increase the presence of spherocrystals and granules of different electron densities, as well as changes in the pattern of glycogen, lipid, and calcium distribution of these cells in the midgut (Cunha et al., 2012).

Conclusion

Conserving natural enemies to improve pest control is a challenge in integrated pest management programs. Thus, the use of Bt plants needs to be evaluated due to its possible adverse effects on non-target organisms. Our results show that different Cry proteins move through the *P. nigrispinus* food chain. Besides, the study provides evidence that exposure period of *P. nigrispinus* females to *S. frugiperda* that consumed maize leaves expressing Bt toxins has no synergistic effects on the non-target species investigated. However, the Cry1F, Cry1A.105, and Cry2Ab2 toxins consumed by *S. frugiperda* cause histopathological changes in the midgut of *P. nigrispinus* (3rd trophic level), which may compromise the predator fitness.

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