Does Cry1Ab maize interfere in the biology and behavioural traits of *Podisus nigrispinus*?

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Abstract

Transgenic plants expressing *Bacillus thuringiensis* proteins can indirectly affect natural enemies of target insects, and the assessment of these effects is essential in risk analysis of genetically modified plants. The biology of *Podisus nigrispinus* (Dallas) (Hemiptera Pentatomidae) and changes in the predation behaviour of this insect when fed with *Bt* (Cry1Ab) maize-fed *Spodoptera frugiperda* (J. E. Smith) larvae (Lepidoptera Noctuidae) was evaluated. The predator showed 43.7% delay in the nymphal development time and 15% biomass reduction in the fifth instar, probably due to low nutritional quality of prey exposed to *Bt* maize. Survival curves were similar in predators fed with *S. frugiperda* larvae exposed and not exposed to *Bt* maize. The predator search time was slightly influenced by the developmental delay of *S. frugiperda* fed with *Bt* maize. In a greenhouse assay *P. nigrispinus* was important in controlling *S. frugiperda* density in *Bt* maize expressing the Cry1Ab protein. Our laboratory results indicate that *Bt* maize may cause indirect effects on *P. nigrispinus*, and suggest that nutritional prey-quality factors other than the *Bt* protein determine the observed negative effects. However, the semi-field greenhouse assays demonstrate that indirect negative effects of *Bt* maize on the predator's performance and search behaviour is not substantiated, and that plant damage is lowest if *Bt* maize is used concurrently with biological control by *P. nigrispinus* for managing *S. frugiperda*.

Key words: *Bt* maize, biological control, predation, *Spodoptera frugiperda*.

Introduction

The use of genetically modified plants that express insecticidal proteins, derived from the soil bacterium *Bacillus thuringiensis* (*Bt*), is increasing worldwide (James, 2013). These plants are an important component of integrated pest management (IPM) systems (Romeis *et al.*, 2008), but in some countries there is skepticism and public concern about a range of issues surrounding transgenic plants, including potential impacts on beneficial organisms (O'Callaghan *et al.*, 2005; Desneux and Bernal, 2010).

Studies on the potential negative effects of *Bt* plants on non-target organisms, in particular predators and parasitoids, have been done to assess the risk involved in their commercial release and post-market monitoring (Naranjo, 2009; Lovei *et al.*, 2009; Lovei and Arpaia, 2005). Possible effects of *Bt* crops on non-target organisms could either be direct or indirect (Shelton *et al.*, 2009; Andow and Zwahlen, 2006; Naranjo, 2005). Direct effects could be due to the toxicity by ingestion of transgenic plant tissues by non-target organisms (e.g., pollen expressing a *Bt* protein) (Schmitz *et al.*, 2003), while indirect effects may occur through multitrophic food chains involving, for example, organisms that feed on other phytophagous insects associated with the transgenic plant (Craig *et al.*, 2008; Conner *et al.*, 2003; Romeis *et al.*, 2006).

Several species of Asopinae stinkbugs are important predators used in biological control. The neotropical species *Podisus nigrispinus* (Dallas) (Hemiptera Pentatomidae) presents a generalist habit and has been con-

sidered a promising biocontrol agent of caterpillar pests in South America (Zanuncio *et al.*, 1996), including Noctuidae family larvae (Mohaghegh *et al.*, 2001). Nymphs and adults of *P. nigrispinus* feed on different prey types but they can also use plants as a supplementary food source, and it is considered an omnivorous species. Studies suggest that feeding on more than one trophic level can complement resources, allowing the predator to survive periods of low prey quality and quantity (Vivan *et al.*, 2003; Gillespie and McGregor, 2000). *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera Noctuidae), an important lepidopteran pest, is endemic in the Americas and causes significant losses in several crops such as maize (*Zea mays* L.), cotton (*Gossypium* spp.), soybean (*Glycine max* (L.) Merrill), rice (*Oryza sativa* L.) and grasses in general (Sparks, 1986).

In Brazil, Cry1Ab maize (MON810 and Bt11) was first commercialized in 2008 (CTNBio, 2014). Since this year, other Cry proteins (Cry1F, Cry1A.105, and Cry2Ab2) and Vip3Aa20 protein against lepidopteran pests were released, and are expressed in our maize hybrids. The target pests of maize that express these proteins are *S. frugiperda*, *Helicoverpa zea* (Boddie) (Lepidoptera Noctuidae), and *Diatraea saccharalis* (F.) (Lepidoptera Crambidae) (Mendes *et al*., 2009). Cry1Ab maize does not express a high dose for *S. frugiperda* (Chilcutt *et al*., 2007), then the use of natural enemies can complement the control of this pest. Natural or applied biological control of *S. frugiperda* by *P. nigrispinus* in areas where *Bt* plants are grown is important in the successful management of this pest and also preserves the use of IPM.

Risk assessment for various natural enemies has been carried out in *Bt* maize expressing the Cry1Ab protein (Naranjo, 2009; Lovei *et al.*, 2009). Studies show that Cry1Ab does not appear to cause direct toxic effects on any of the predator and parasitoid groups examined (for review see Romeis *et al.*, 2006). Lynch *et al.*(1999) confirmed the adverse effects of "Bt11 event" sweet maize (Cry1Ab protein) on the development of *S. frugiperda* in field experiments. Cry1Ab maize affected survival, developmental times, and growth rates of *S. frugiperda* larvae and its parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera Braconidae) (Ramirez-Romero *et al.*, 2007). Cunha *et al.* (2011) showed that *P. nigrispinus* can acquire Cry1Ac protein by feeding on *S. frugiperda* larvae, which might affect predation ability. These effects can affect the prey-quality, which can indirectly affect the predator. In addition to direct effects, indirect effects on natural enemies cannot be ignored and must be considered as part of a risk assessment of *Bt* crops.

Because of this complex interaction and the uncertainty about the protein exposure level, studies are needed to clarify the impact of *Bt* maize on Hemiptera predators. Our aim with this work was to evaluate possible prey-mediated effects of Cry1Ab maize on the predatory insect, *P. nigrispinus*.

Materials and methods

Insect rearing

The insects were reared in the Ecotoxicology and Insect Management laboratory of EMBRAPA Milho e Sorgo, Brazil (25 ± 2 °C, 60 ± 10% RH, 14:10 L:D). The colony of *P. nigrispinus* was started with adults collected in the field and was kept for more than four years in the laboratory, with an average population of 150 insects in every generation. In the 1st instar the insects were reared only with a honey and distilled water (10%) solution. From the 2nd instar, the nymphs were reared on *S. frugiperda* larvae. The larvae provided for the predator were from a colony reared on artificial diet (Greene *et al.*, 1976) for more than 10 years in the laboratory, and in the absence of exposure to any insecticide.

Plant growing conditions for laboratory bioassays

The plants used were MON810 event (Cry1Ab) of Pioneer 30F35 hybrid and its near isogenic non-*Bt* maize hybrid (control). The maize was planted, weekly, in the experimental field of EMBRAPA Milho e Sorgo. Each planting consisted of a plot with five rows of 20 m and five plants/meter. Cultivation was performed according to the recommendations for Brazil (Cruz, 2010) without any application of insecticides, fungicides and herbicides. Manual weeding was performed.

Laboratory experimental conditions

The experiments were conducted in the Ecotoxicology and Insect Management laboratory of EMBRAPA Milho e Sorgo, Brazil (25 ± 2 °C, 60 ± 10% RH, 14:10 L:D). For the bioassays, 2nd instar nymphs of *P. ni-*

grispinus were used, because from this stage onwards the nymphs of this predator adopt a predatory habit (Zanuncio *et al.*, 1996). Only water was given to 1st instar nymphs, through cotton wool. After hatching, part of the *S. frugiperda* neonates (≈ 2,000) were fed with Cry1Ab maize leaves and the other part (≈ 500) with leaves of the near isogenic non-*Bt* maize hybrid (control). The larvae were reared with *Bt* and non-*Bt* maize leaves until the 4th instar. To feed the neonates, maize leaves were used between stages 13 and 16 (Lancashire *et al.*, 1991). The leaves of both maize genotypes (*Bt* and non-*Bt*) were offered in a volume of approximately 50 cm^3 and set out in 50 mL plastic cups. The leaves were changed every 48 hours to prevent dryness.

Performance of predators fed with prey exposed to *Bt* and non-*Bt* maize in the laboratory

One hundred 2nd instar *P. nigrispinus* nymphs were randomly separated into two groups of 50, and were individually placed in plastic cups (50 mL). One group of nymphs was fed with *S. frugiperda* larvae fed with Cry1Ab maize and the other group was fed with larvae that had fed on non-*Bt* maize (control). No plant material was offered to the predator. The nymphs were fed *ad libitum* daily with 2nd to 4th instar larvae of *S. frugiperda*, according to the treatment. It was observed that Cry1Ab maize-fed *S. frugiperda* larvae were smaller, so it was necessary to provide larger quantities of these larvae. In each cup a cotton ball dampened with water was placed and changed every two days, for moisture and water supply. This bioassay assessed the duration of each instar, the survival in each nymphal instar and of the total nymphal stage, and in addition, the weight of the 5th instar nymphs using an analytical scale. Twenty-six insects that were fed with the prey exposed to *Bt* maize were weighed and 26 were weighed of the non-*Bt* maize. The weight assessment was performed on the first day of the 5th instar, because in this stage presents the highest biomass gain (Zanuncio *et al.*, 1996). Molting was verified through the exuviae that were in the cups after each molt. The data were recorded daily. Adults were not evaluated due to their low number.

Searching time of the predator for prey exposed to *Bt* and non-*Bt* maize in the laboratory

A factorial experiment in a completely randomized design was conducted with 3 and 7 day-old *S. frugiperda* larvae, reared with *Bt* or non-*Bt* maize leaves (Cry1Ab), and provided for 2nd and 4th instars of *P. nigrispinus* nymphs and adults, in a total of 12 treatments. Ten larvae from each treatment were size measured to assess their development. The first instar nymphs were put individually in Petri dishes of 5 cm (diameter) until they reached the second instar and were left with only a cotton ball dampened with distilled water. *S. frugiperda* larvae were confined in Petri dishes and a predator was released in the center of each plate, while the larvae were kept near the edges of the Petri dishes. We took care to keep the distance between the prey and the predator the same during this release. Nineteen to 31 individuals (= replicates) of *S. frugiperda* per treatment

were used depending on larvae availability. The larvae were used only once in each replicate. The duration from release of the predator in the center of the Petri dish to the first capture of a larva (searching time) was timed using a chronometer.

Greenhouse bioassay

The bioassay was installed in a greenhouse on April 22nd, 2013 at EMBRAPA Milho e Sorgo, Brazil (25 ± 5 °C, 70 ± 15% RH) in a completely randomized design. The insects used were from the laboratory colony and the plants were the same as those mentioned before: MON 810 event (Cry1Ab) of Pioneer 30F35 hybrid and its near isogenic non-*Bt* maize hybrid (control). There were four treatments with 12 pots in each: two different plant genotypes infested with *S. frugiperda*, and plants infested with *P. nigrispinus* and not infested. Three maize plants were planted in pots (20 L) with soil as substrate and fertilized with 50 g of NPK 08-28-16 and 0.3% Zn/100 kg. The plants were soaked daily (500 mL of water). All the plants were manually infested with five *S. frugiperda* neonates when they were in the V6 stage (6 leaves). The pots containing the plants were covered with a screen net and a metallic support was used to sustain it. Five days after the larva infestation half of the plants from each treatment (6 pots) were infested with five *P. nigrispinus* 2nd instar nymphs (to ensure predation) and the other half were not infested. The total number of *S. frugiperda* and *P. nigripinus* per treatment were 180 of each one (12 pots × 3 plants × 5 insects). *S. frugiperda* injuries on the plants were graded using a scale of 0 (zero) to 5 (five), where 0- corresponds to plants without injury; 1- plants with scraped leaves; 2- plants with perforated leaves; 3- plants with injuries on leaves and stem; 4- plants with almost destroyed stem; and 5- plants with many leaves and stem totally destroyed. The injuries were evaluated 7, 15 and 21 days after larva infestation. At the 21st day after larva infestation, the number of pupae removed from the pot soil was also evaluated.

Statistical analyses

Homogeneity of variances was tested with a Bartlett test before the statistical analysis for all the trials. The results of predator mortality throughout their development were subjected to survival analysis using the nonparametric LIFETEST procedure (SAS Institute, 2002), in which survival curves are obtained using Kaplan-Meyer estimators. The datasets of nymphal stage duration were transformed to $(x + 1)^{0.5}$ and were analyzed with a one-way analysis of variance (ANOVA), which was performed for each instar separately. The other parameters assessed were not transformed and were analyzed with a one-way ANOVA. A factorial ANOVA was used to determine effects of *S. frugiperda* larval ages (3 and 7 days), maize genotypes (Cry1Ab and control), and predator stages (2nd and 4th instar, and adult) on predator searching time. Differences between means in each maize genotype were tested using Fisher's protected least significant difference. All analyses were done using JMP software (SAS Institute, 2002) at $\alpha = 0.05$. *S. frugiperda* injury (x) of greenhouse experiment were transformed to arcsin $\sqrt{x}/100$ to the normality adjustment. Subsequently, the data were submitted to analysis of variance and means were compared by Tukey test ($P \leq 0.05$).
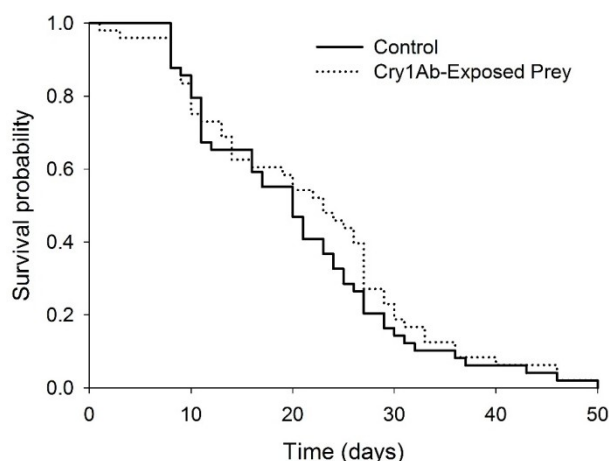

Figure 1. Survival plots of *P. nigrispinus* reared on control and Cry1Ab-exposed larvae of *S. frugiperda*. The survival curves were not significantly different ($\chi^2 = 0.06$, df = 1, $P = 0.799$).

Results

Performance of predators fed with prey exposed to *Bt* and non-*Bt* maize in the laboratory

To assess the effects of *S. frugiperda* fed with *Bt* maize on *P. nigrispinus*, we measured nymph development, survival and biomass. Independently of feeding on exposed or non-exposed prey, *P. nigrispinus* nymph survival was statistically not different as evidenced by the comparison of the survival curves (figure 1). The percentage of predators that reached adulthood also did not differ between treatments ($F_{1, 97} = 1.03$, $P = 0.312$). For individuals fed with prey exposed to *Bt* maize, the average was 18.0 ± 5.5% (mean ± SE) and for individuals that fed on non-*Bt* maize (control) it was 26.5 ± 6.4%.

The predator's nymphal development was faster (i.e., had shorter duration) on non-*Bt*-fed prey than on those exposed to the *Bt* maize, which was evident for both the duration of each instar and for total nymphal stage duration (second instar to adult) (table 1). This represents a delay of 43.7% in the predator development time by feeding on prey reared with *Bt* maize. In addition, 5th instar *P. nigrispinus* fed with *Bt*-fed *S. frugiperda* accumulated 15% lower biomass than predators fed with prey not exposed to *Bt* maize. The weight of *P. nigrisrinus* that fed on *Bt*-fed *S. frugiperda* was 19.1 ± 0.91 mg, and the weight of *P. nigrispinus* that fed on non *Bt*-fed *S. frugiperda* was 22.5 ± 1.27 mg ($F_{1,51} = 4.57$; $P = 0.037$).

Searching time of the predator for prey exposed to *Bt* and non-*Bt* maize in the laboratory

To assess if the *Bt* maize intake by *S. frugiperda* would disrupt *P. nigrispinus* capture of this prey, we measured the searching time of the predator. When we

Table 1. Nymphal stage development duration of *P. nigrispinus* when fed with *S. frugiperda* larvae reared on *Bt* and non-*Bt* maize.

Treatment (prey supply)	Nymphal stage development duration (days)				Total nymphal development (days)
	Second	Third	Fourth	Fifth	
Cry1Ab maize	3.4 ± 0.07a (*n* = 48)	5.95 ± 0.15a (*n* = 37)	5.51 ± 0.36a (*n* = 31)	10.19 ±1.02a (*n* = 26)	27.44 ± 1.21a (*n* = 9)
Non *Bt* maize	3.09 ± 0.01b (*n* = 49)	2.83 ± 0.22b (*n* = 37)	3.72 ± 0.30b (*n* = 33)	6.04 ± 0.50b (*n* = 27)	15.46 ± 0.27b (*n* = 13)

Mean ± SE followed by the same letter in the column are not significantly different by ANOVA ($P \leq 0.05$). The *n* indicates the number of individuals of each nymphal stage that were used in the ANOVA of the total of 50 that started the experiment.

took the average of the searching time for 3 and 7 day-old larvae we observed that only for the 4th instar nymphs was there a difference ($P < 0.0005$) between searching time for *S. frugiperda* fed with Cry1Ab maize (8.08 ± 1.55 min) and those fed with control maize (3.47 ± 0.67 min) (figure 2). The differences in searching time for 2nd instar nymphs and adults are not significant. Note that the larger the predator, the longer searching time was. There was no significant interaction ($F_{2,261} = 2.02$; $P = 0.135$) when the three factors were assessed (larva age, maize genotype, and predator stage), and when larva age and the maize cultivar were assessed ($F_{2,261} = 2.32$; $P = 0.128$). The interaction was significant between the maize genotype and predator stage ($F_{2,261} = 2.13$; $P = 0.046$), and between the predator stage and larva age ($F_{2,261} = 18.25$; $P < 0.0001$).

Greenhouse bioassay

To assess the effects of *Bt* maize on *P. nigrispinus* biological control of *S. frugiperda*, we evaluated the injury done by the herbivore on the *Bt* and non-*Bt* plants in the presence and absence of its predator, and the number of pupae. *P. nigrispinus* controlled its prey on non-*Bt* maize, as we can see through the damage scale, which was significantly different after the seventh day ($P < 0.001$) (figure 3). *Bt* maize itself controlled *S. frugiperda*, but the infestation of *P. nigrispinus* significantly reduced the injury caused by its prey on this maize until the 22nd day ($P < 0.001$). The number of pupae at the end of this bioassay was drastically reduced by the infestation with *P. nigrispinus* in both Cry1Ab maize and non-*Bt* maize. For the "non-*Bt*" treatment the number of pupae was 37; for the "non-*Bt* + Podisus" it was 2; for the "Cry1Ab" it was 17, and for "Cry1Ab + Podisus" it was 3. Note that overall plant injury was not different after 15 and 22 days except for in the non-*Bt* treatment. However, the number of pupae at the end of the experiment show that the treatments with *P. nigrispinus* drastically reduced the larval survival of *S. frugiperda* in the *Bt* and non-*Bt* treatments.

Discussion and conclusions

There was no direct effect of the exposure of *S. frugiperda* to *Bt* maize on the *P. nigrispinus* predator. The survival of the predator fed with *Bt* maize-fed *S. frugiperda* was not different to that fed with non-*Bt*
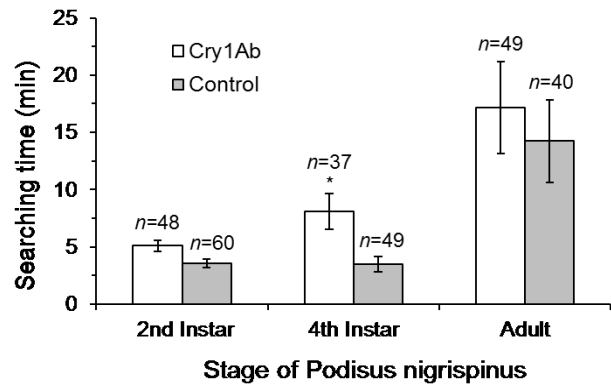
Figure 2. Average search time in minutes (± standard error) of *P. nigrispinus*, at three different stages (2nd and 4th instars, and adult) when 3- and 7-day old larvae of *S. frugiperda* fed with *Bt* maize and control (non-*Bt* maize) were offered as prey. The results were concatenated, because there was no interaction between the larva age and the maize cultivar ($F_{2,261} = 2.32$; $P = 0.128$). The *n* indicates the number of replicates tested in each treatment. The presence of the asterisk (*) indicates a statistical difference between the treatments within each stage of the predator (Fisher's LSD).
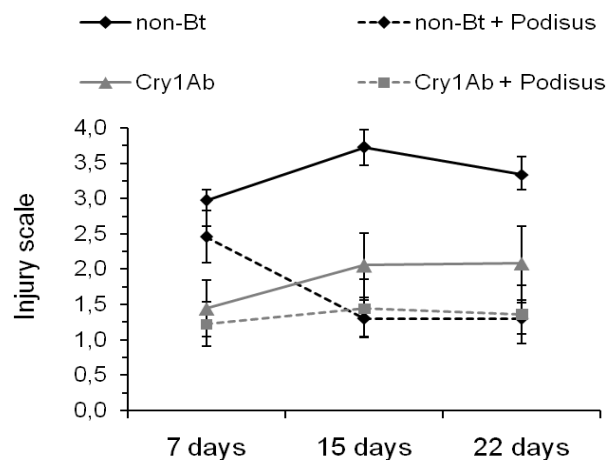
Figure 3. Injury scale (mean ± standard error) of *S. frugiperda* larvae on Cry1Ab maize and non-*Bt* maize in the presence or absence of its predator *P. nigrispinus* after 7, 15 and 22 days of larvae infestation in the greenhouse.

maize-fed larvae. The survival to adult was low, 18% and 26.5%, respectively. We observed that these results were due to *S. frugiperda* attacking *P. nigrispinus* when the nymphs were molting. The same was observed for the percentage of predators that reached adulthood. Nonetheless, the nymphal stage development time was faster and the predator had greater body mass accumulation when it was fed with prey not exposed to *Bt* maize. It is likely that these results are related to the poor quality of prey exposed to *Bt* maize as food for the predator. We did not quantify Cry1Ab ingestion and contents in the prey and predator, but we observed that larvae that fed with *Bt* maize developed more slowly than those fed with non-*Bt* maize. This slow development demonstrates a sublethal effect in *S. frugiperda*, which may have decreased its nutritional quality. Developmental delay was observed in *Spodoptera littoralis* (Boisduval) (Lepidoptera Noctuidae) when fed with Cry1Ab maize (Vojtech *et al.*, 2005). The purified Cry1Ab protein affected survival, developmental times, and growth rates of *S. frugiperda* larvae, while Cry1Ab *Bt*-maize tissue affected the parasitoid *C. marginiventris* development times, adult size, and fecundity. In addition, low biomass accumulation can be another indicator of lower nutritional quality. For *S. frugiperda*, Mendes *et al.* (2011) reported a 20-fold reduction in the weight of larvae fed with Cry1Ab maize compared to those fed with the same non-*Bt* hybrid.

Studies using *Bt*-resistant lepidopteran larvae, the hosts for parasitic wasps, support such indirect adverse effects (Schuler *et al.*, 2003; Schuler *et al.*, 2004; Ferry *et al.*, 2006; Chen *et al.*, 2008). In the case of predatory arthropods, the most prominent example of putative prey-mediated effects is that of the green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera Chrysopidae). Dutton *et al.* (2002) and Hilbeck *et al.* (1998) reported a longer larval development and reduced survival of *C. carnea* fed with *S. littoralis* and *Ostrinia nubilalis* (Hubner) (Lepidoptera Crambidae) larvae, which were fed with Cry1Ab maize. The authors concluded that *C. carnea* survival was not compromised directly as a result of the predator's susceptibility to the *Bt* protein, but that this resulted from eating sublethally affected (sick) prey. The mechanisms responsible for these presumed indirect effects are not clear, but they might be caused by changes in the nutritional quality of sublethally affected larvae. These indirect effects, mediated by the prey quality, have been reported in other studies with *Bt* transgenic plants (Ashouri *et al.*, 2001; Couty *et al.*, 2001; Bernal *et al.*, 2002; Bell *et al.*, 2003). Therefore, the predominant factor for the delay in predator development in this study can be attributed to the prey quality when fed with *Bt* maize.

In general, the searching time of the predator was not affected by *S. frugiperda* larvae fed with Cry1Ab maize. The only significant difference found was due to the predator age. Only in the 4th instar did the predator take a longer time to find *Bt*-fed larvae. We suppose that this is because of the slower development of *S. frugiperda* when fed with *Bt* maize. Three-day-old *S. frugiperda* larvae fed with *Bt* and non-*Bt* maize measured 2 ± 0.5 mm and 2 ± 0.8 mm (mean $\pm$ SE), respectively; and

7-day-old larvae measured 6 ± 1.3 mm and 8 ± 0.9 mm, respectively. The 2nd instar predators are small, so we believe that the developmental delay of *S. frugiperda* did not confuse them when searching out the prey. Similarly, adults did not differentiate between larvae fed with *Bt* maize or not, because both preys were very small relative to the size of the predator. But 4th instar predators, which were of medium size, could differentiate the developmental delay of the prey when fed with *Bt* maize.

Note that the larger the predator, the longer it took to find the prey. The adult predator had more difficulty in finding the larvae. This must be due to the lower perception of small larvae by the adult predator. It was observed that the predator often passed over the prey without noticing it. Vieira *et al.* (1997) found similar results when studying the searching time of the predator *Scymnus* (*Pullus*) *argentinicus* (Weise) for *Schizaphis graminum* (Rondani) (Hemiptera Aphididae). They found that the search time increased gradually with the age of the predator. We hypothesize that this would not affect the consumption of prey by *P. nigrispinus* in the field, since suitable stages of the predator and the prey occur concurrently.

The greenhouse assay showed that the integration of *Bt* maize with biological control by *P. nigrispinus* is important for successfully managing *S. frugiperda*. Malaquias *et al.* (2014) found similar results working with *Bt* cotton leaves expressing Cry1Ac. They observed that the type of functional response by *P. nigrispinus* was not affected by the cotton cultivar (*Bt* or non-*Bt* cotton). A study by Chilcutt *et al.* (2007) indicated that Cry1Ab maize lines did not provide high efficacy against *S. frugiperda*. This clearly suggests that Cry1Ab maize does not express a "high dose" against this pest as defined by the EPA (2001). Despite Cry1Ab maize do not control alone *S. frugiperda*, this *Bt* maize was registered in Brazil to protect the crop against *S. frugiperda*, *H. zea*, and *D. saccharalis*. This study indicates that *P. nigrispinus* may contribute as a biocontrol agent of *S. frugiperda* larvae in *Bt* maize fields without side effects to predator. Transgenic crops and biological control can act as complementary tactics in IPM (Romeis *et al.*, 2008).

In summary, an indirect effect of *Bt* maize was detected in laboratory studies with *P. nigrispinus* mediated by sublethal toxicity of the Cry1Ab protein to *S. frugiperda* larvae. This was evident in the longer developmental period observed and lower predator biomass, which is probably due to the inferior quality of the prey fed with *Bt* maize. However, the percentage of *P. nigrispinus* that reached adulthood was not influenced by feeding with *S. frugiperda* reared on *Bt* maize. Therefore, it cannot be inferred how this indirect effect would affect the predator's population growth and impact on the target insect. Future studies should be done to try to detect direct effects using the purified Cry1Ab protein. The searching time of *P. nigrispinus* for *S. frugiperda* larvae was little influenced by the prey development when fed with Cry1Ab maize. The greenhouse bioassays demonstrate that indirect negative effects of *Bt* maize on the predator's performance and search behaviour is not sub-

stantiated under semi-field conditions, and that plant damage is lowest if *Bt* maize is used concurrently with biological control by *P. nigrispinus* for successfully managing *S. frugiperda*.

Acknowledgements

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