

Bt cotton and the predator *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) in the management of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) resistance to lambda-cyhalothrin

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Abstract This study sought to provide relevant information for developing effective programs to manage *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) resistance to insecticides by quantifying and describing the interactions between cotton Bt and *Podisus nigrispinus* (Dallas) (Heteroptera; Pentatomidae) in the management of lambda-cyhalothrin resistance in *S. frugiperda* fed Bt cotton leaves expressing Cry1Ac (Bollgard, Monsanto, St Louis, MO, USA). These effects were assayed by studying functional responses. Third instar *S. frugiperda* larvae were used for the following treatments: strains resistant (1) or susceptible (2) to lambda-cyhalothrin fed Bollgard cotton leaves and strains resistant (3) or susceptible (4) to lambda-cyhalothrin fed non-Bt cotton leaves. The predatory behavior of *P. nigrispinus* females was best represented by a sigmoid curve. The type of *P. nigrispinus* functional response was not affected by the cotton cultivar or *S. frugiperda* strain used in the trials. *P. nigrispinus* females invested a greater amount of handling time (T_h) in *S. frugiperda* larvae that were susceptible to insecticides and fed non-Bt cotton ($T_h = 1.72$ h) compared to those from the insecticide-resistant strain fed with Bt cotton ($T_h = 1.23$ h) or even compared to those that were lambda-cyhalothrin resistant and fed Bt ($T_h = 1.17$ h) or non-Bt cotton

($T_h = 1.17$ h). The results in the present study can be applied in the development of management programs targeting *S. frugiperda* resistance to pyrethroid lambda-cyhalothrin. The integration of Bt cotton concurrent with biological control by the Asopinae *P. nigrispinus* is important for successfully managing *S. frugiperda* resistance to lambda-cyhalothrin.

Keywords Biological control · Transgenic cotton · Resistance management · Lambda-cyhalothrin · Fall armyworm

Genetically modified crops offer new options for increasing the availability of food and sources of raw materials used by the textile industry in developing countries. Bt cotton was first employed in Brazil in 2005 with the approval of Bollgard cotton (Barros 2012). The transgenic Bt cotton cultivar, which harbors and expresses the Cry1Ac gene from the bacterium *Bacillus thuringiensis* Berliner, is toxic to the lepidopterans *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae), *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) and *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), which are members of a group of defoliating and fruit larvae affecting Brazilian cotton production (Huesing and English 2004). Other genetically modified cultivars that confer resistance to insects have also been employed commercially in Brazil, including Bollgard II (Monsanto), which expresses Cry1Ac and Cry2Ab2 (Colli 2011), WideStrike (Dow AgroSciences), which expresses pyramided Cry1F and Cry1Ac (Adamczyk et al. 2008), and TwinLink (Bayer), which contains the cry1Ab and cry2Ae genes (Fok 2011). Recently, the Brazilian National Technical Commission on Biosafety approved the use of the Glytol × Twinlink

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(Bayer) (Wilkinson and Ford 2007) and MON 15985 \times MON 88913 (Randhawa and Chhabra 2013) cultivars for both controlling defoliating lepidopteran larvae and improving weed management. Because transgenic cotton should reduce insecticide application, it can also be used for managing insect resistance to conventional insecticides (Wu et al. 2005).

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) is a polyphagous migratory pest, which is endemic to the Western Hemisphere and can attack more than 80 plant species, including maize (*Zea mays* Linné), cotton (*Gossypium hirsutum* Linné), sorghum [*Sorghum bicolor* (Linné) Moench], rice (*Oryza sativa* Linné), millet [*Penisetum americanum* (Linné) Bulrush], peanut (*Arachis hypogaea* Linné), alfalfa (*Medicago sativa* Linné), and other cultivated and wild plant species (Knippling 1980; Pashley 1986). It is typically controlled using insecticides based on the characteristics of its attacks (Diez-Rodríguez and Omoto 2001). There is a substantial gene flow in the animal populations collected from the cotton crop in Midwest Brazil, and a low variability is observed between populations collected on this crop. A correlation between the population structure and the cotton crop has not been detected (Martinelli et al. 2007). Nonetheless, different *S. frugiperda* populations have suffered constant selection pressures from insecticides used in various agricultural systems (Yu 1992; Leibe and Capinera 1995), which may have contributed to the establishment of populations resistant to several classes of insecticides in Brazil, such as pyrethroids (Diez-Rodríguez and Omoto 2001). The inheritance of resistance to the pyrethroid lambda-cyhalothrin by *S. frugiperda* is autosomal recessive (Diez-Rodríguez and Omoto 2001). The evolution of resistance tends to be delayed when inheritance is recessive (Roush and McKenzie 1987). However, as resistance reaches a high frequency, the restoration of susceptibility in the absence of selective pressure can be slower because the resistant individuals that survive the application of an insecticide would all be homozygous (Roush and McKenzie 1987).

The presence of effective natural enemies of pests in agroecosystems is one of the bio-ecological factors that should be considered for managing insecticide resistance. Transgenic cotton can positively affect generalist predator insects because indiscriminate pesticide applications can be reduced, thus increasing the population density of these natural enemies (Hagerty et al. 2005; Romeis et al. 2008; Lu et al. 2012). Defoliating lepidopteran larvae are an important group of pests that reside in cotton crops, and outbreaks of these pests are commonly associated with the occurrence of Pentatomidae predators (Pereira et al. 2010). These predators feed on a variety of prey species, which greatly contributes to the control of different insect pests (Ramalho 1994). The generalist predator *Podisus*

nigrispinus (Dallas) (Heteroptera: Pentatomidae) could be potentially employed for biologically controlling several Lepidoptera species that thrive in Brazilian cotton crops, including the fall armyworm, *S. frugiperda* (Zanuncio et al. 2008). The predatory ability of *P. nigrispinus* is an important attribute that should be considered in management programs targeting defoliating lepidopteran larvae (Pereira et al. 2008).

Functional responses are used to assess the potential of natural enemies to control insect pests in various situations (Faria et al. 2004; Madadi et al. 2011). Predator–prey dynamics are directly influenced by the relationship between the available prey density and the number of prey attacked, which is a determining factor in the performance of predators in biological control systems (Holling 1959). An increase in prey availability can lead to increased predator consumption because the opportunities for encounter and attack will increase over time until satiation occurs (O’Neil 1990). The functional response of *P. nigrispinus* to *S. frugiperda* depends on the defensive capability of this prey species as well as its density (Zanuncio et al. 2008). Little is known about the functional responses of the neotropical predator *P. nigrispinus* to prey that develop on transgenic host plants and to prey larval strains that are resistant to this control tactic. Information describing the predatory or parasitic behavior of natural enemies for different pest genotypes (resistant or susceptible) is important for designing strategies to manage pest resistance to insecticides (Liu et al. 2012). This study sought to provide relevant information for developing effective programs to manage *S. frugiperda* resistance to insecticides by quantifying and describing the interactions between Bt cotton and *P. nigrispinus* in the management of lambda-cyhalothrin resistance in *S. frugiperda* fed Bt cotton leaves expressing Cry1Ac (Bollgard, Monsanto, St Louis, MO, USA). These effects were assayed by studying functional responses.

Materials and methods

Insects and cotton cultivars

The effects of Bt and non-Bt cotton (*G. hirsutum*) cultivars together with *S. frugiperda* strains susceptible or resistant to the pyrethroid lambda-cyhalothrin on the functional responses of *P. nigrispinus* were quantified. The *S. frugiperda* populations used in this study were derived from a breeding stock maintained in the Laboratory of Insect Resistance to Control Tactics (Laboratório de Resistência de Insetos a Táticas de Controle—LRITC) at the Luiz de Queiroz School of Agriculture (Escola Superior de Agricultura “Luiz de Queiroz”—ESALQ), University of São

Paulo (Universidade de São Paulo—USP), Piracicaba, São Paulo (SP), Brazil. The larvae were maintained in glass tubes (2.5 cm in diameter by 8 cm in height) containing an artificial diet based on bean, wheat germ and beer yeast. The *S. frugiperda*-resistant strain was obtained through selection pressure applied at the LRITC. Newly emerged *P. nigrispinus* female adults were obtained from breeding colonies maintained at the Biological Control Unit of Embrapa Algodão, Campina Grande, Paraíba, Brazil. These insects were maintained at 26 ± 1 °C under 70 ± 10 % RH and a 12-h photophase. The temperature and photoperiod conditions were based on the recommendations of Medeiros et al. (2004) and Malaquias et al. (2009, 2010), respectively.

One genetically modified Bollgard cultivar (DP 404 BG), which harbors a synthetic version of a gene from *B. thuringiensis* that encodes the Cry1Ac endotoxin, and another cultivar that lacked this endotoxin (cultivar DP-4049) were used. The cultivars were supplied by Monsanto through an agreement between the company and the LRITC. The cultivars were planted in a greenhouse without controlled climatic conditions in plastic buckets (10-l capacity). An organic substrate mixed with topsoil at a 1:1 ratio was used. The material was free of insecticide and/or acaricide. The leaves were collected from the top third of the plants in the vegetative phase, and plants that had flowered were discarded.

Two types of prey, corresponding to third-instar *S. frugiperda* larvae that were either resistant (I) or susceptible (II) to lambda-cyhalothrin, were used. These larvae were fed during their first and second instars on the aforementioned cotton cultivars (Bt and non-Bt cotton). The larvae utilized in the experiments were all active. The following experimental treatments were performed: *S. frugiperda* larvae that were resistant (1) or susceptible (2) to the pyrethroid were fed Bollgard (DP 404 BG) cotton leaves, and *S. frugiperda* larvae that were resistant (3) or susceptible (4) to lambda-cyhalothrin were fed non-Bt cotton leaves (cultivar DP4049). During the first and second instars, the *S. frugiperda* larvae received their food in transparent plastic vials (200-g capacity).

Insect bioassays

Newly emerged *P. nigrispinus* female adults were fasted for 24 h and were then individually transferred to PVC arenas (30 cm high, 27 cm diameter) covered with an organza-type fabric. A leaf collected from the upper third portion of a cotton plant (approximately 2 months old) was placed in the center of each arena in a glass container. The arenas were maintained at 26 ± 1 °C, 70 ± 10 % RH and a 12-h photophase.

At 24-h intervals, each *P. nigrispinus* female was fed third-instar *S. frugiperda* larvae that were either resistant or susceptible to lambda-cyhalothrin and had been fed leaves from Bt or non-Bt cotton plants during the first to third instars at densities of 1, 2, 4, 8 and 16 larvae per leaf in each arena. Arenas with the same larvae densities were used to assess the larvae mortality in the absence of the predator to control for mortality due to cannibalism and the transgenic plant. To determine the predatory capacity of *P. nigrispinus* females as a function of the treatments, larvae exhibiting tegument lesions, a lack of mobility or whose body contents had been partially or completely removed by the predator were considered predated.

We employed a factorial experiment arranged in randomized blocks, which included the two cotton cultivars and two prey types (lambda-cyhalothrin susceptible and resistant), and five replicates were performed for each density. The same female was repeatedly provided the same number of larvae at 24-h intervals (1, 2, 4, 8 or 16 larvae).

Data analysis

To determine the type of functional response that was observed, predation data from two trials were subjected to multinomial logistic regression analysis by using PROC GENMOD (SAS Institute 2006). Holling's (1959) equation was used to estimate the following parameters: the attack rate (a) when a type II response was observed (Eq. 1), the constant (b) when a type III response was detected (equation 2) and the handling time (T_h) for both cases (types II and III). For the type II model, the following equation was used:

$$N_e = aNT / (1 + aNT_h) \quad (1)$$

where N_e is the number of predated larvae; N is the number of larvae offered; a is the attack rate; T is the total time available for the predator to eat the prey; T_h is the handling time. In some cases, the attack rate increased linearly ($a = bN$, where b is a constant), which resulted in a type III model of the functional response. Thus, the resulting model was a reduced type III equation (Juliano 1993)

$$N_e = bN^2T / (1 + bN^2T_h) \quad (2)$$

The parameters handling time (T_h), attack rate (a) and constant (b) were estimated via nonlinear regression (least squares method) using PROC NLIN (SAS Institute 2006). The coefficient of determination (R^2) of nonlinear models cannot be calculated as in linear models, $R^2 = 1 - (\text{SQR}/\text{SQT})$, because most nonlinear models do not have an identifiable intercept. In this case, SAS uses the uncorrelated sum of squares instead of the total sum of squares (Freund and Littell 1986). The R^2 values of these models

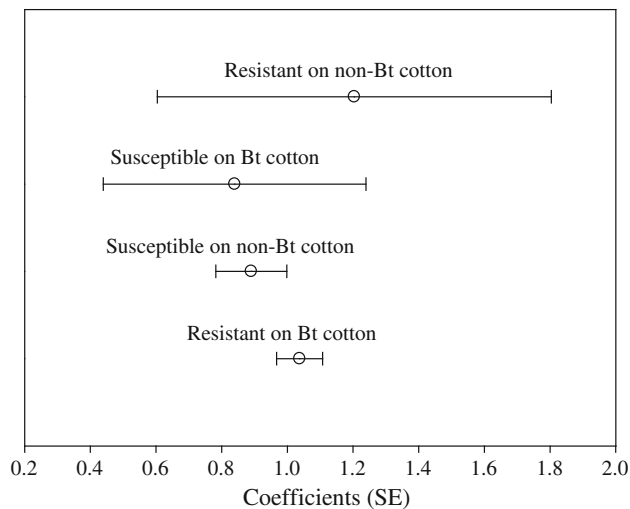


Fig. 1 Results of polynomial logistic regression analyses indicate the estimates and standard errors of linear coefficients for the proportion of prey killed from two strains of *Spodoptera frugiperda* by *Podisus nigrispinus* females relative to the initial prey numbers on Bt and non-Bt cotton

were calculated as $R^2 = 1 - (S_y^2 / S_{td}^2)$, where S_y^2 is the variance of the model residuals and S_{td}^2 is the variance of the observed means for consumption rates.

Results

In the absence of predators, independent of the tested density, the survival of third instar larvae of *S. frugiperda* was greater than 98 %. Therefore, it was not necessary to perform a correction for mortality due to cannibalism or the Bt plants. The sign of the linear coefficient from the polynomial logistic regression analysis was used to distinguish the shape of the functional response curve, with negative and positive values indicating type II and III responses, respectively (Juliano 1993). The results demonstrated that the predatory behavior of *P. nigrispinus* was best represented by the type III functional responses because, regardless of the treatment, the value of the linear coefficient was positive (Fig. 1). Thus, predatory behavior of *P.*

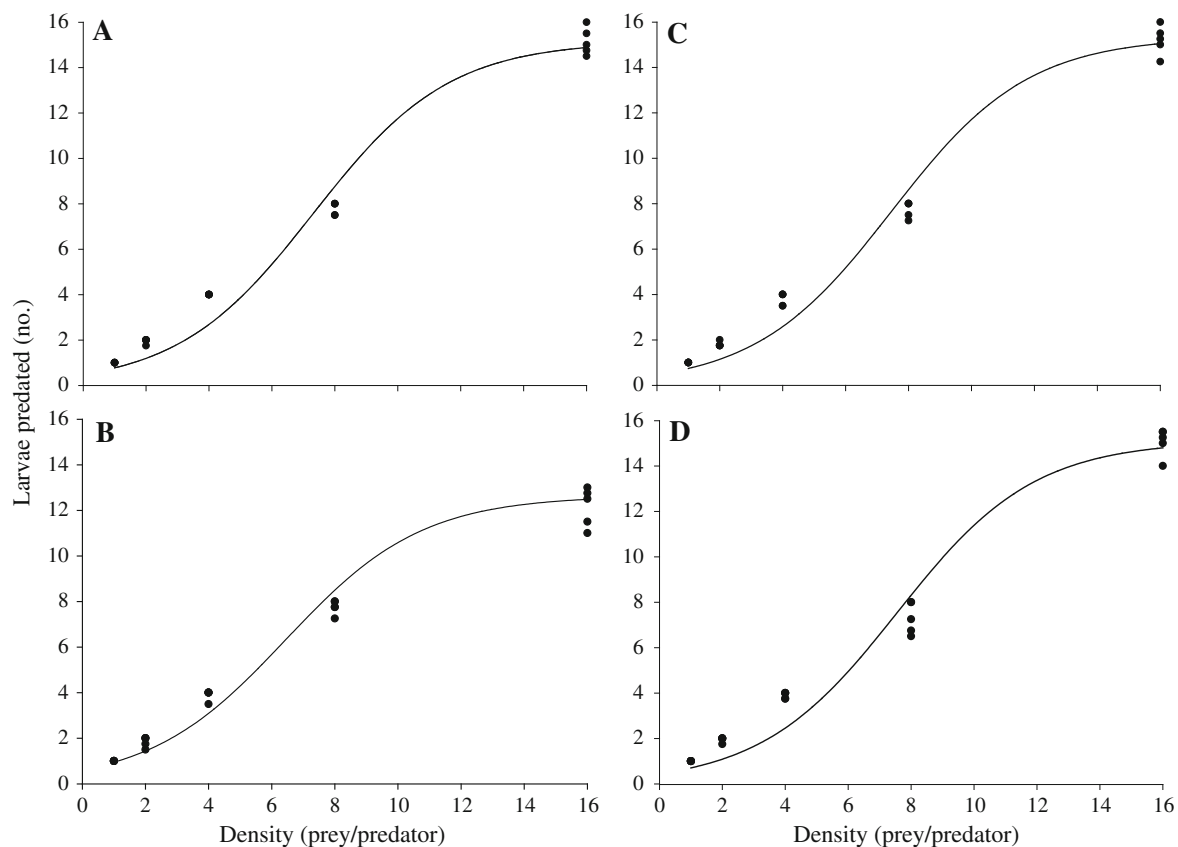


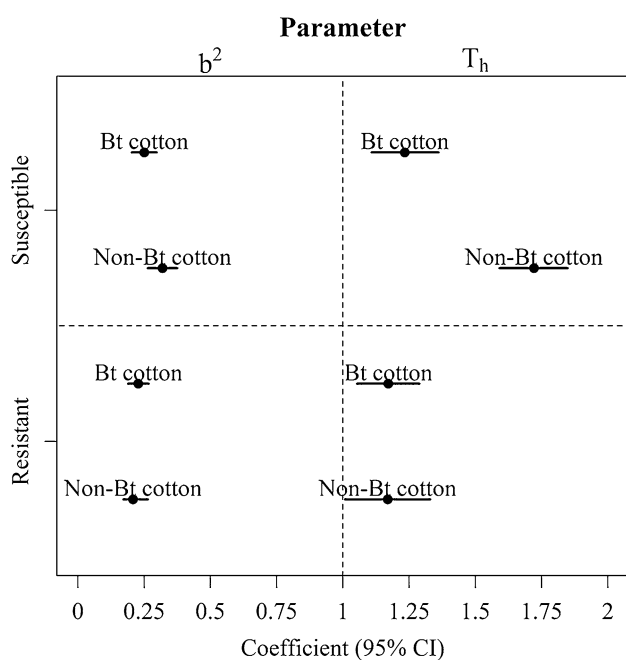
Fig. 2 Functional response of *Podisus nigrispinus* females to different densities of *Spodoptera frugiperda* larvae that were either susceptible to lambda-cyhalothrin on Bt cotton (a) and non-Bt cotton

(b) or resistant to lambda-cyhalothrin on Bt cotton (c) and on non-Bt cotton (d). Data observed (points) and predicted (lines) for model of type III functional response

Table 1 The mean constant “*b*” and handling time (T_h) values (confidence interval) estimated by the disc equation, indicating a type III functional response of *Podisus nigrispinus* females to larvae of two *Spodoptera frugiperda* strains on Bt and non-Bt cotton

Strain/cultivar	b^2 (95 % CI)	T_h (h) (95 % CI)	R^2	$P > F$
<i>S. frugiperda</i> susceptible to lambda-cyhalothrin				
Bt cotton	0.25 (0.20–0.30) a	1.23 (1.11–1.36) b	0.97	0.0001
Non-Bt cotton	0.32 (0.26–0.38) a	1.72 (1.59–1.85) a	0.96	0.0001
<i>S. frugiperda</i> resistant to lambda-cyhalothrin				
Bt cotton	0.23 (0.19–0.27) a	1.17 (1.05–1.29) b	0.98	0.0001
Non-Bt cotton	0.21 (0.17–0.26) a	1.17 (1.01–1.33) b	0.96	0.0001

Values followed by the same letter (within a column) are not significantly different at the 5 % level when the 95 % confidence intervals of the estimates overlap

**Fig. 3** The mean constant “*b*” and handling time (T_h) values (confidence interval) estimated by the disc equation, indicating a type III functional response of *Podisus nigrispinus* females to larvae of *Spodoptera frugiperda* resistant on Bt ($R^2 = 0.98$) and non-Bt cotton ($R^2 = 0.96$) and susceptible on Bt ($R^2 = 0.97$) and non-Bt cotton ($R^2 = 0.96$). Values followed by the same letter are not significantly different at the 5 % level when the 95 % confidence intervals of the estimates overlap

nigrispinus irrespective of cotton cultivar and *S. frugiperda* strain was best represented by a sigmoid curve (Fig. 2).

The values of the constant “*b*” obtained from the Holling’s disc equation applied to the susceptible strain of *S. frugiperda* on Bt and non-Bt cotton were 0.25 and 0.32, respectively, whereas values of 0.23 and 0.21 were obtained for the lambda-cyhalothrin resistant on Bt and non-Bt cotton, respectively. Thus, the attack rate of female *P. nigrispinus* preying on *S. frugiperda* larvae increased linearly (a-bN) irrespective of cotton cultivar and *S. frugiperda* strain. Furthermore, there was no significant

difference in this variable “*b*” among the studied conditions because there was an overlap in the confidence intervals (95 % CI) (Fig. 1).

The confidence intervals did not overlap between the handling time (T_h) observed in female *P. nigrispinus* offered *S. frugiperda* larvae susceptible to lambda-cyhalothrin and previously fed non-Bt cotton compared to the other treatments. Therefore, female *P. nigrispinus* invested more handling time (T_h) in *S. frugiperda* larvae that were susceptible to lambda-cyhalothrin and fed non-Bt cotton ($T_h = 0.172$ h) compared to those from the lambda-cyhalothrin-resistant strain fed with Bt cotton ($T_h = 1.23$ h) or even compared to those that were lambda-cyhalothrin resistant and fed Bt ($T_h = 1.17$ h) or non-Bt cotton ($T_h = 1.17$ h) (Table 1 and Fig. 3).

Discussion

The effectiveness of a predator is partly related to the type of its functional response in the field, and information obtained from laboratory experiments may be used to infer the basic mechanism of the predator-prey interactions under field conditions (Houck and Strauss 1985).

The type of functional response can be determined based on the sign of the linear coefficient, which is negative for type II and positive for type III (Juliano 1993). The findings of our study revealed that the predatory behavior of *P. nigrispinus* was best represented as a type III functional response, irrespective of cotton cultivar and *S. frugiperda* strain (Fig. 1).

Prey density dependence related to the predation of *A. argillacea* has been recorded in *P. nigrispinus* by Pereira et al. (2010). The functional response of predators may change from one type to another under different environmental conditions (Mohaghegh et al. 2001). According to Hassell (1978), the type of functional response of a given predator can change depending on the prey type. This change in response may be due to changes in the predator foraging behavior when exposed to a particular food source. Predators exhibit three types of functional responses (Holling 1959), and only type III yields a density-

dependent mortality (Murdoch and Oaten 1975). The density-dependent mortality factors could regulate the population (Nicholson 1933) and stable equilibrium densities, and population oscillation damping would most likely occur with a type III rather than a type II functional response.

Researchers have similarly postulated that a predator with a type III functional response could regulate prey density more effectively than a predator with a type II functional response (Murdoch 1969, Murdoch and Oaten 1975). One postulated mechanism for type III functional responses is the concentration of predator hunting efforts in high-density patches (Hertlein and Thorarinsson 1987).

Predator responses as a function of prey density that are represented by a sigmoidal curve (type III) are crucial for programs that employ biological control agents because the predator can regulate the prey population through this type of behavior, even when the prey are dense (Murdoch and Oaten 1975).

The acquisition of more prey by the predator *P. nigrispinus*, especially with highly dense prey, implies a higher attack rate, which represents the prey-searching efficiency of this predator. The attack rate was a function of prey density ($a = bN$), irrespective of cotton cultivar and *S. frugiperda*; however, there was a longer handling time (T_h) observed in predator females offered susceptible larvae that had previously been fed on non-transgenic cotton (Table 1).

The handling time parameter estimated in this study differs from those estimated by Zanuncio et al. (2008) for *P. nigrispinus* preying on *S. frugiperda* larvae. This difference is most likely related to the type of arena, densities, temperature and prey strains employed in these studies. Larvae that are susceptible to insecticides and fed non-Bt cotton are thought to exhibit higher nutritional quality (Ramalho et al. 2011) relative to larvae from the other experimental conditions, e.g., *S. frugiperda* larvae resistant to lambda-cyhalothrin and fed Bt cotton. According to Vivan et al. (2003) and Laycock et al. (2006), *P. nigrispinus* females require a longer handling time (T_h) for higher nutritional quality prey, i.e., they invest more time in capturing, attacking and ingesting this prey. Although the Bollgard cultivar does not cause a high mortality rate in *S. frugiperda*, this cultivar affects qualitative nutritional parameters, such as the production of feces and the rates of food metabolism and assimilation (Ramalho et al. 2011).

The results in the present study can be applied in the development of management programs targeting *S. frugiperda* resistance to pyrethroid lambda-cyhalothrin. Therefore, the integration of Bt cotton concurrent with biological control by the Asopinae *P. nigrispinus* is important for successfully managing *S. frugiperda* resistance to lambda-cyhalothrin. However, other factors should

be considered in future studies because the functional response of an insect predator at different prey densities, in addition to being mediated by the interaction between these two trophic levels, may also be affected by morphological and biochemical traits of host plants, especially with zoophytophagous insects (De Clercq et al. 2000).

In conclusion, the results of our study revealed that (1) Bt cotton expressing Cry1Ac (Bollgard) and the resistance of *S. frugiperda* larvae to lambda-cyhalothrin does not alter the type of functional response observed in *P. nigrispinus*, as indicated by the sign of the linear coefficient (positive for type III) (Juliano 1993), (2) the predatory behavior of *P. nigrispinus* is best represented by a type III functional response, irrespective of cotton cultivar and *S. frugiperda* strain (Fig. 3), and (3) female *P. nigrispinus* exhibited a higher handling time (T_h) for *S. frugiperda* larvae susceptible to lambda-cyhalothrin and fed non-Bt cotton.

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