

How predation by *Podisus nigrispinus* is influenced by developmental stage and density of its prey *Alabama argillacea*

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Abstract

The functional response of a predator to the density of its prey is affected by several factors, including the prey's developmental stage. This study evaluated the functional response of *Podisus nigrispinus* (Dallas) (Hemiptera: Heteroptera: Pentatomidae) females to fourth instars and pupae of *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae), an important pest of cotton (*Gossypium hirsutum* L., Malvaceae) in Brazil. The prey were exposed to the predator for 12 and 24 h, and in densities of 1, 6, 12, 18, 24, and 30 items per predator female. The predation data were subjected to polynomial regression logistic analysis to determine the type of functional response. Holling and Rogers' equations were used to estimate parameters such as attack rate and handling time. *Podisus nigrispinus* females showed functional response types II and III by preying on larvae and pupae, respectively. The attack rate and handling time did not differ between the 12 and 24 h exposure times. Predation rate was higher at higher larval and pupal densities; predation was highest at a density of 30 prey items per female, and it was similar at 18 and 24 prey per predator. Understanding the interaction of predators and their food resources helps to optimize biological control strategies. It also helps the decision-making and the improvement of release techniques of *P. nigrispinus* in the field.

Introduction

Alabama argillacea (Hübner) (Lepidoptera: Noctuidae) is considered the main defoliating pest of cotton (*Gossypium hirsutum* L., Malvaceae) in Brazil (Nascimento et al., 2011; Ramalho et al., 2011). In the Brazilian South-Central region, it is considered a late pest (Oliveira et al., 2008); however, in the Northeast region, except for the State of Bahia, this pest attacks cotton plants during their early stages and may occur sporadically when the cotton crop reaches its maturity stage (Ramalho, 1994). Both, as an early and as a late pest it causes considerable production losses (Michelotto et al., 2014).

The control of cotton infestations by *A. argillacea* is done by the constant application of insecticides (Ramalho et al., 2011). However, the frequent use of these synthetic

chemical insecticides in cotton agroecosystems increases the production costs and causes undesirable effects on non-target organisms, such as the reduction in populations of natural enemies (predators and parasitoids) of the focal pest (Costa et al., 2010; Silva et al., 2011).

Biological control of *A. argillacea* by means of the stink-bug *Podisus nigrispinus* (Dallas) (Hemiptera: Heteroptera: Pentatomidae) is an effective alternative to reduce the damage caused by this pest (Malaquias et al., 2014). The predator *P. nigrispinus* shows high ability to search and attack its prey (Malaquias et al., 2014, 2015). *Podisus nigrispinus* is able to survive under food scarcity conditions (Ramalho et al., 2008) and shows versatility by consuming alternative food sources (Malaquias et al., 2010). This predator is found in agroecosystems of the Brazilian cotton-producing states; therefore, it is an important natural enemy of cotton leafworm (Medeiros et al., 2004).

Quantitative knowledge of the predator-prey interaction, including a predator's response to prey density (functional response) and prey handling time, is extremely

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important for the implementation of applied biological control programs (Bale et al., 2008). The functional response of *P. nigrispinus* has been assessed in various cotton agroecosystems (Malaquias et al., 2015). Factors such as temperature (Malaquias et al., 2015), plant species (De Clercq et al., 2000), and plant physiology (Carneiro et al., 2010) influence the natural enemy's predation behavior. In addition, the developmental stages of both the predator (Hassanpour et al., 2011; Madadi et al., 2011) and the prey (Farhadi et al., 2010) are known to affect the attack efficiency of this biological control agent.

The prey's aggressiveness to the predator's attack is an important feature that should be taken into consideration in bio-ecological studies on pentatomid predators (Azevedo & Ramalho, 1999; Silva et al., 2012). Differential prey defense among developmental stages can cause variability in the predator's foraging behavior (Azevedo & Ramalho, 1999). Foraging predators are vulnerable to the risk of exposure, therefore they require strategies to minimize a prey's defensive action. Knowledge of the interactions of the predator *P. nigrispinus* and its primary prey *A. argillacea* in cotton is still incipient. Lepidoptera larvae display defensive responses to predator attacks, thus they may hinder predation success (Silva et al., 2012). On the other hand, the movement of these larvae may be considered a stimulus to the attack by their natural enemies, in comparison to the immobile pupal stage. *Podisus nigrispinus* is able to change its predatory behavior depending on the food density or availability (Ramalho et al., 2008; Pereira et al., 2010).

This study aimed to study *P. nigrispinus* predation using *A. argillacea* larvae and pupae as prey and to assess the predator's functional response to densities of these prey stages. We hypothesized that (1) the various developmental stages (larva and pupa) of *A. argillacea* cause variability in *P. nigrispinus* predatory behavior, (2) under free-choice condition, *P. nigrispinus* females prey more on pupae than on larvae, and (3) under no-choice condition, *P. nigrispinus* females prey equally on larvae and pupae.

Materials and methods

Location, insects, and cotton cultivars

The rearing of *P. nigrispinus* and *A. argillacea* as well as the bioassays were done in the Biological Control Unit of Embrapa Cotton, Campina Grande, PB, Brazil. *Podisus nigrispinus* nymphs and adults as well as *A. argillacea* larvae from stock rearings were kept in BOD-type climatic chambers at 25 °C, 70 ± 10% r.h., and L12:D12 photoperiod. *Alabama argillacea* larvae were fed on cotton leaves (*G. hirsutum*, BRS Safira cultivar). The cultivar was planted in plastic pots (20 cm diameter, 30 cm high) and

placed in a greenhouse at 35 ± 10 °C, 70 ± 10% r.h., and L12:D12 photoperiod. Predator nymphs and adults were fed housefly larvae (*Musca domestica* L.).

Bioassays

Fourth instars of *A. argillacea* and adult female predators (6–8 days old), that had presumably copulated, were used in the bioassays. These females were subjected to fasting (no prey) for 24 h. The bioassays were conducted at 28 °C, 70 ± 10% r.h., and L12:D12 photoperiod. Plastic arenas (21 cm diameter and 1.8 cm high disposable Petri dishes) were used, each with eight cotton leaf discs (cv. BRS Safira, 10 cm diameter) laying on a layer of moist cotton wool. The leaf discs were equidistantly distributed from the center of the arena and used as food for the prey. Each dish received 16 prey items, i.e., all discs in a dish received either two larvae or two pupae (no-choice assays) or the discs received alternately two larvae or two pupae (free-choice assays).

The study used a randomized complete block design with two treatments (larva and pupa) distributed in 10 replications. An adult female predator was released in the center of each arena and the killed prey were counted 12 and 24 h after the beginning of the bioassay. Each female predator was weighed before and 24 h after the beginning of each bioassay. Larvae showing lesions in their integument, lack of mobility, or body content partially or completely sucked by the predator were considered preyed. Pupae were considered preyed if they were partially or completely sucked.

Foraging behavior

The foraging behavior of *P. nigrispinus* females was observed in free-choice and no-choice assays, at 20-min intervals, during a 6-h period. We recorded the time the predator spent (1) at rest (Tr), (2) seeking the prey (Tlp), (3) dominating the prey (from prey encounter to beak insertion in the prey's body) (Td), (4) feeding (from beak insertion until removal) (Tf), and (5) looking for a new prey (from beak removal to beak insertion in the body of a new prey) (Tnp).

Functional response

Fourth instars and pupae of *A. argillacea* were offered during a 24-h period to each *P. nigrispinus* female at the following densities: 1, 6, 12, 18, 24, and 30 larvae or pupae per cotton leaf disc (10 cm diameter). The prey killed by each female predator were counted after 12 and 24 h. Ten replications were performed for each prey density and prey type (larvae or pupae) combination and a new predator female was used in each replication. Larvae showing lesions in the integument or lack of mobility, and larvae

and pupae showing body content partially or completely sucked by the predator were considered preyed. There was no prey mortality in the absence of the predator; therefore, it was not necessary to make any correction for natural mortality. Each female predator was weighed before and 24 h after the beginning of the bioassay. This is important to determine the prey density effects on the predator's weight gain.

Data analysis

The numbers of killed larvae and pupae in the free-choice and no-choice assays were subjected to variance analysis (Proc GLM; SAS Institute, 2006), after testing for normality (Kolmogorov–Smirnov) (Stephens, 1979) and homo-scedasticity (Bartlett, 1937). The mean numbers of killed prey were compared by F test ($\alpha = 0.05$). The female predator weight gain or loss (initial weight – final weight) was analyzed using Proc REG (SAS Institute, 2006), considering weight the dependent variable and prey density and prey type (larva or pupa) independent variables.

Predation data were subjected to polynomial regression logistic analysis to determine the type of functional response: the linear coefficient signal is negative for functional response type II and positive for functional response type III. Data regarding the ratio of killed larvae or pupae were adjusted to the binomial distribution (SAS Institute, 2006). We used the following logistic model:

$$N_a/N_0 = \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3) / [1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)], \quad (1)$$

where N_0 is the prey density, N_a is the number of prey killed, and P_0 , P_1 , P_2 , and P_3 are the logistic regression parameters associated with the curve slope. The equations by Holling (1959) and Rogers (1975) were used to estimate the attack rate and handling time parameters.

Holling's disc equation. Holling's equation (1959) was used to estimate the following parameters: attack rate (a) when type II (equation 2) and constant (b) when type III (equation 3) was used, and handling time (T_h) in both types II or III. For a type II model, the following equation was used:

$$N_e = aNT / (1 + aNT_h), \quad (2)$$

where N_e is the number of killed larvae, N is the number of offered larvae, and T is the total time available for the predator to feed on the prey. In some cases, the attack rate increased linearly ($a = bN$), which resulted in a type III functional response:

$$N_e = bN^2T / (1 + bN^2T_h). \quad (3)$$

Rogers' model. The 'random' functional response equation by Rogers is the integral of Holling's equations over time, depending on the successive prey densities (Rogers, 1972). For a type II functional response, the integration of Holling's equation results in the random predator equation:

$$N_e = N_0 \{1 - \exp[a(T_h N_e - T)]\}, \quad (4)$$

where N_0 is the initial number of prey. For a type III functional response, the reduction in the number of prey depends on the constant rate b , which is a function of the preys' initial (N_0) or current (N) densities (Hassell et al., 1977; Hassell, 1978). The simplest equation can be found when b is a function of the initial prey density:

$$N_e = N_0 \{1 - \exp[(d + bN_0)(T_h N_e - T) / (1 + cN_0)]\}. \quad (5)$$

Parameters such as handling time (T_h) and attack rate (a) and the constants b , c , and d were estimated by nonlinear regression (least squares method) using Proc NLIN (SAS Institute, 2006). As the determination coefficient (R^2) of non-linear models does not present an identifiable intercept, SAS uses the sum of uncorrelated squares instead of the sum of total squares. The R^2 values of these models were calculated as $R^2 = 1 - (S^2_y / S^2_{td})$, in which S^2_y is the variance of the model residues and S^2_{td} the variance of the observed means.

A Generalized Linear Model (GLM) was used to test the significance of the main and interaction effects of the fixed variables 'prey stage', 'assessment time', and 'prey density' (SAS Institute, 2006). The means were compared using the Student–Newman–Keuls test ($\alpha = 0.05$).

Results

Free-choice and no-choice bioassays

Podisus nigrispinus females preferred *A. argillacea* larvae over pupae in the free-choice assays. i.e., within 6 h the *P. nigrispinus* female preyed on an average of six larvae and only one pupa ($F_{1,9} = 78.55$, $P < 0.0001$; Figure 1). However, when the predator was not offered a choice of prey, there was no difference between the average numbers of larvae and pupae killed (six vs. eight; $F_{1,9} = 0.66$, $P = 0.44$; Figure 1).

Foraging behavior

When female *P. nigrispinus* was offered the choice between *A. argillacea* larvae vs. pupae for 6 h, they preyed

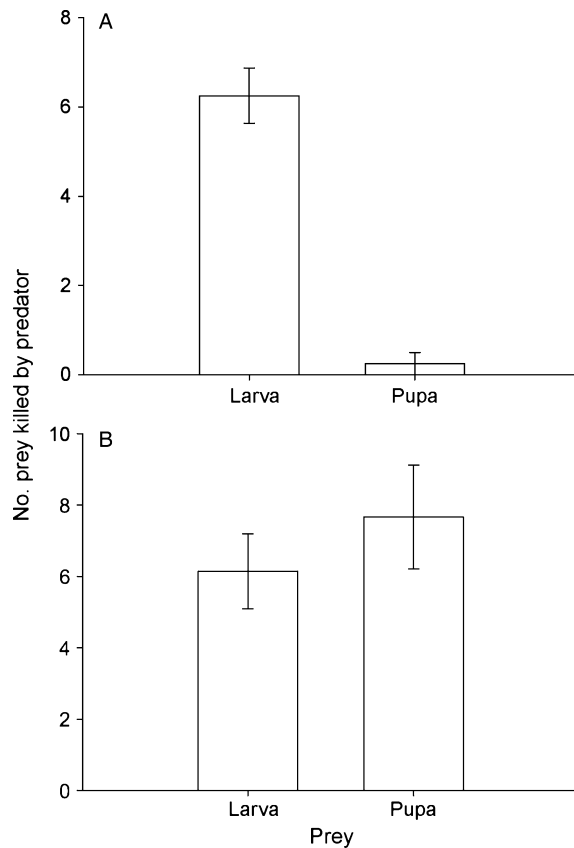


Figure 1 Mean ($\pm$ SE) number of *Alabama argillacea* larvae and pupae predated by *Podisus nigrispinus* during 6 h of exposure in (A) choice and (B) no-choice bioassays ($n = 10$ females per treatment).

exclusively on larvae and spent an average of 76.6% (4.6 h) of their time feeding on them (Figure 2A). On the other hand, when *P. nigrispinus* females did not have a choice, they spent on average 90.5% (5.4 h) and 17.0% (1.0 h) of their time preying on larvae and pupae, respectively (Figure 2B). Thus, *P. nigrispinus* females that had a choice of prey consumed their preferred prey (larvae) 50 min faster.

We observed the predatory behavior of 10 *P. nigrispinus* females preying on *A. argillacea* larvae and pupae at 20-min intervals over a period of 6 h in total. It appeared that the female completely consumed each prey item before starting on the next, independent of prey density. They did not kill prey items simultaneously, not even at higher prey densities.

Functional response

When *P. nigrispinus* females had *A. argillacea* larvae as prey, predation stabilized at 18–24 larvae per arena (Figure 3). However, with pupae as prey, predation stabilized

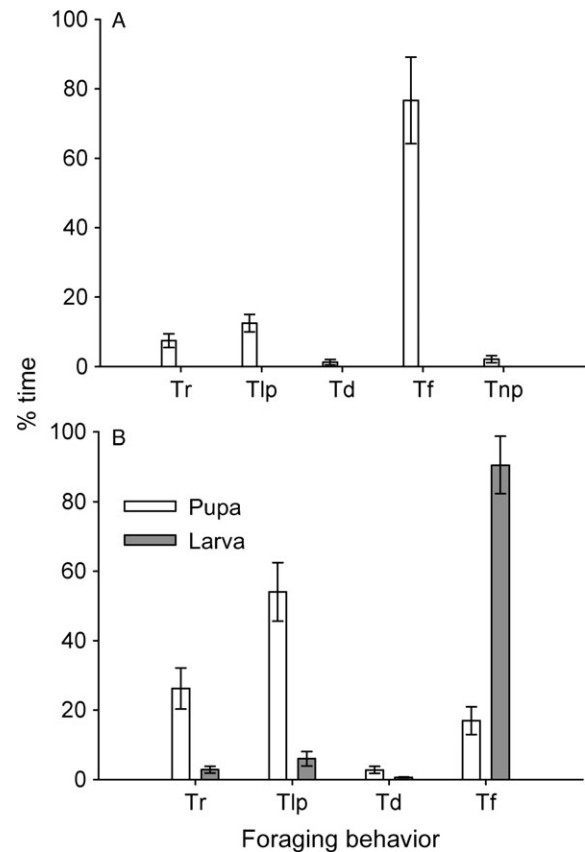


Figure 2 Mean ($\pm$ SE) time expenditure (% of 6-h observation time) by *Podisus nigrispinus* females foraging on larvae and pupae of *Alabama argillacea* in (A) choice and (B) no-choice bioassays, according to the following behavior types: Tr = predator sits still and does not feed, Tlp = predator searches prey, Td = predator dominates prey (from prey encounter to insertion of the beak in the prey's body), Tf = feeding on prey (from insertion until withdrawal of the beak), and Tnp = predator searches new prey (from withdrawal of the beak until encounter of another prey).

at 10–12 pupae per arena (Figure 3). These data indicate that *P. nigrispinus* need to consume more larvae than pupae in order to get satiated. The extraction of food from pupae appeared more difficult, as the female predators' weight gain was lower when they consumed pupae (Figure 3). This matches the average number of prey items consumed in the 24-h period, as a function of prey density, which ranged from 1.10 to 9.12 larvae and 0.87 to 3.12 pupae (Figure 4). The predator's weight gain was lower at low vs. high prey (both larvae and pupae) densities (Figure 3). Incidentally, *P. nigrispinus* females lost weight when they were subjected to densities of one or six pupae per female (Figure 3).

Podisus nigrispinus predatory behavior was affected by the prey's developmental stage, as the linear coefficient

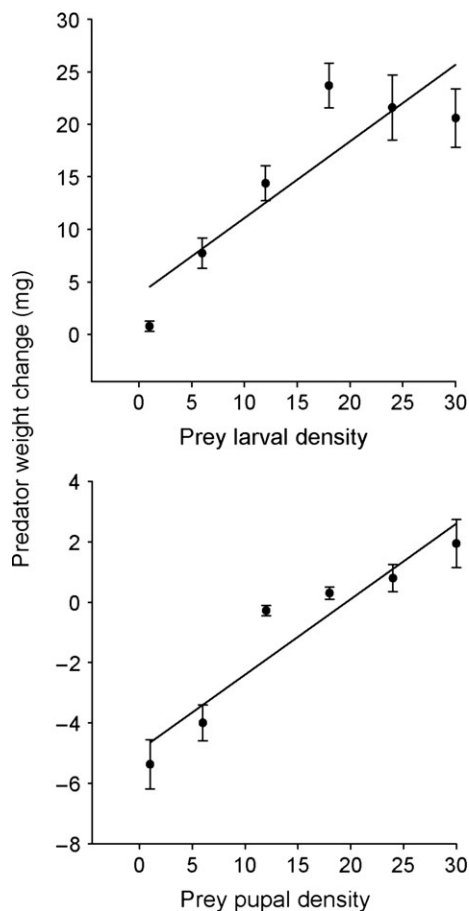


Figure 3 Mean ($\pm$ SE) weight change by *Podisus nigrispinus* females during feeding assays in which they were exposed to different densities of *Alabama argillacea* larvae ($y = 0.79 + 0.73x$, $R^2 = 0.78$; $F_{1,4} = 14.17$, $P = 0.020$) or pupae ($y = -4.90 + 0.25x$, $R^2 = 0.89$; $F_{1,4} = 33.21$, $P = 0.0045$).

signal was negative in *A. argillacea* larval stage, regardless the assessment time (functional response type II) (Figure 5). On the other hand, functional response type III better described *P. nigrispinus* predation of *A. argillacea* pupa i.e., the value of the linear coefficient was positive (Figure 5). These results indicate that the type of functional response of *P. nigrispinus* is affected by the prey's developmental stage.

The average numbers of *A. argillacea* larvae killed by *P. nigrispinus* females increased with increasing larval densities, featuring an asymptotic regression curve (Figure 4). The residual sum of squares (RSS), Akaike information criterion (AIC), and determination coefficient (R^2) values indicated good adjustments of the Holling's models for functional response type II with larval-stage prey at both 12-h (RSS = 1.1031, AIC = 0.0367, $R^2 = 0.9476$, $F_{2,4} = 253.76$, $P < 0.0001$) and 24-h intervals (RSS =

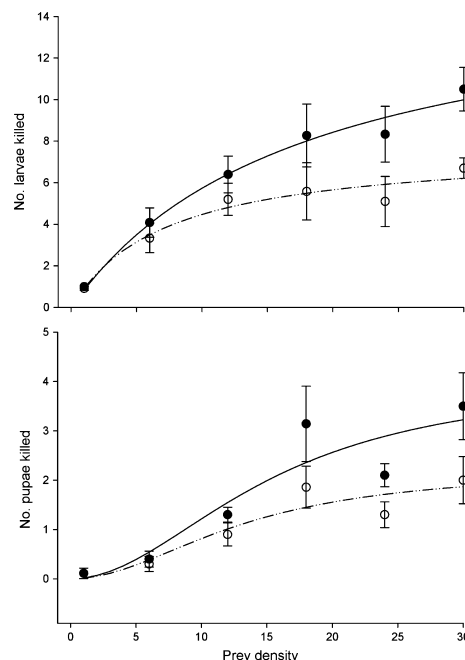


Figure 4 Predation rate of *Podisus nigrispinus* females in relation to the density of larvae and pupae of *Alabama argillacea* after 12 h (—) and 24 h (---). Note the different scales on the vertical axes.

1.0104, AIC = 0.0038, $R^2 = 0.9827$, $F_{2,4} = 605.19$, $P < 0.0001$), and for type III with pupal-stage prey at 12-h (RSS = 0.3810, AIC = 3.079, $R^2 = 0.8758$, $F_{2,4} = 50.76$, $P = 0.0014$) and 24-h intervals (RSS = 1.4033, AIC = 9.83, $R^2 = 0.8572$, $F_{2,4} = 38.48$, $P = 0.0024$) (Table 1). No good adjustment of Rogers' model was found in *P. nigrispinus* functional response when they had *A. argillacea* larvae or pupae as prey, except for the larval stage at the 24-h interval (RSS = 4.6754, AIC = 0.5141, $R^2 = 0.9201$, $F_{2,4} = 129.23$, $P = 0.0002$; Table 1).

The *P. nigrispinus* females' attack rate represented by the constants a (larvae) and b (pupae) did not significantly differ between the assessment periods of 12 ($a = 0.0887$, $b = 0.00107$) and 24 h ($a = 0.0374$, $b = 0.000722$), as the 95% confidence intervals overlapped. The handling time (T_h) of larvae by female predators did not significantly differ from that of pupae, at neither of the two assessment intervals, although female predators relatively invested more time in pupae than in larvae (Table 2).

Predation differed among densities ($F_{5,212} = 48.78$), prey stages ($F_{1,212} = 242.37$), and time ($F_{1,212} = 17.49$, all $P < 0.001$). The effect of *A. argillacea* stage did not depend on density and assessment time because the interaction involving these factors was not significant ($F_{5,212} = 0.03$, $P = 1.0$), nor were the interactions of density*assessment

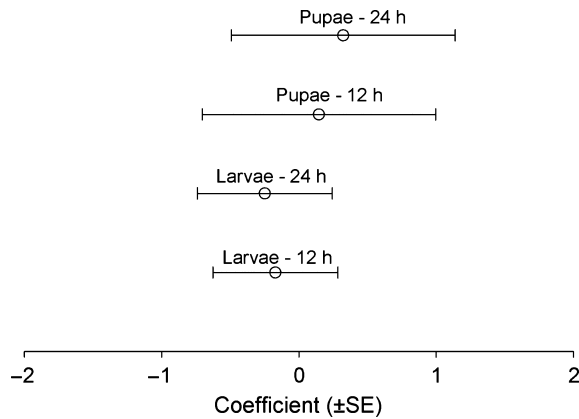


Figure 5 Estimates ($\pm$ SE) of linear coefficients for polynomial logistic regression analyses of the proportion of prey killed (larvae or pupae) of *Alabama argillacea* by *Podisus nigrispinus* females after 12 and 24 h.

time ($F_{5,212} = 1.47$, $P = 0.20$), stage*assessment time ($F_{1,212} = 0.25$, $P = 0.32$), and density*stage ($F_{5,212} < 0.01$, $P = 1.0$). Predation increased with increasing prey density and the increase appeared to level off at ca. 12 insects per *P. nigrispinus* female (Figure 6A). More larvae than pupae were killed (5.3 larvae vs. 1.3 pupae; Figure 6B). Thus, the developmental stage of *A. argillacea* influenced the behavior of *P. nigrispinus*. The mean number of prey items (larvae + pupae) killed by a predator female at 12 and 24 h of exposure was 2.9 and 4.1 (Figure 6C).

Discussion

Models involving the non-replenishment of prey are able to provide estimates for stochastic predation. However, if the experiments are conducted with prey replenishment, such replenishment should be incorporated into the statistical analysis using Holling's disc equation to accurately validate the findings (Juliano, 1993). As for the bioassays conducted without prey replenishment, the use of models

is questionable due to the incorrect estimation of the functional response curve. Although Rogers' model is well known since 1972, inappropriate methods are used. One of the reasons for this to happen is the ease of using Holling's model in relation to Rogers' model (Juliano, 1993). However, the use of both models requires a good fit to the observed data. The sum of squared residuals (SSR), the determination coefficient (R^2), and the AIC are commonly used to assess the fit of the models (Haghani et al., 2009). A good model should contain high R^2 values and low SSR values (Roy et al., 2002; Kontodimas et al., 2004). Although the current study was conducted without prey replenishment, good fit of Rogers' model was not found in the functional response of *P. nigrispinus* females to *A. argillacea* larvae and pupae in the 12-h period, only for the larvae in the 24-h period. One of the difficulties in using Rogers' model lies on the estimation of parameters b, c, and d, which, as an aside, were not significant when incorporated into the model related to the current study data. This may occur due to the reduced degree of freedom for error and the inevitable correlations between the estimated parameters (Juliano, 1993). Zamani et al. (2006) analyzed the functional response of parasitoids *Aphidius colemani* Viereck and *Aphidius matricariae* Haliday and found better adjustments of Holling's model in comparison to Rogers' model, using R^2 values.

The type of functional response by *P. nigrispinus* females was affected by the prey's developmental stage. Functional response type III best represents predation by *P. nigrispinus* on *A. argillacea* pupae, whereas predation on larvae is best represented by type II. This study results indicate that prey age and density influence the female predator's functional response curve. Temperature conditions (Mohaghegh et al., 2001), host plant features (De Clercq et al., 2000), and insecticides (Malaquias et al., 2014) caused variation in the types of functional response. Predation behavior may also be influenced by the prey's defense capability, and higher predation was especially

Table 1 Regression analyses for two models of the functional response of *Podisus nigrispinus* to densities of *Alabama argillacea* larvae and pupae after 12 and 24 h

Model	Stage/time (h)	RSS	CSS	AIC	R^2	F	d.f.	P
Rogers'	Larva/12	8.7368	21.06	0.7225	0.5851	30.29	2,4	0.0038
type II	Larva/24	4.6754	58.57	0.5141	0.9201	129.23	2,4	0.0002
Rogers'	Pupa/12	1.0383	3.07	0.0140	0.6617	4.34	4,2	0.19
type III	Pupa/24	3.6792	9.83	0.4885	0.6257	3.36	4,2	0.24
Holling	Larva/12	1.1031	21.06	0.0367	0.9476	253.76	2,4	<0.01
type II	Larva/24	1.0104	58.57	0.0038	0.9827	605.19	2,4	<0.0001
Holling	Pupa/12	0.3810	3.07	-0.3618	0.8758	50.76	2,4	0.0014
type III	Pupa/24	1.4033	9.83	0.1270	0.8572	38.48	2,4	0.0024

RSS, residual sum of squares; CSS, corrected sum of squares; AIC, Akaike information criterion.

Table 2 Estimated mean ($\pm$ SE; 95% confidence intervals in parentheses) attack rate (coefficients a and b) and prey handling time (Th) for *Podisus nigrispinus* females preying on larvae and pupae of *Alabama argillacea* after 12 and 24 h, based on Holling's disc equation and Rogers' random equation

Stage/time (h)	Holling's disc equation		Rogers' random equation		Th (h)
	a	b	a	b	
Larva/12 h	0.0887 $\pm$ 0.0253 (0.0183–0.1591)	–	0.0531 $\pm$ 0.0281 (–0.0250 to 0.1312)	–	1.0896 $\pm$ 0.4926 (–0.2779 to 2.4572)
Larva/24 h	0.0374 $\pm$ 0.00571 (0.0216–0.0533)	–	0.0430 $\pm$ 0.0113 (0.0116–0.0743)	–	1.3798 $\pm$ 0.3150 (0.5053–2.2542)
Pupa/12 h	–	0.00107 $\pm$ 0.00057 (0.0–0.00265)	–	0.00708 $\pm$ 1.9796 (–8.5106 to 8.5247)	0.0725 $\pm$ 19.6543 (–84.49 to 84.63)
Pupa/24 h	–	0.000722 $\pm$ 0.00042 (0.0–0.00189)	–	0.0847 $\pm$ 1.3245 (–5.6140 to 5.7834)	0.0422 $\pm$ 130.30 (–560.60 to 560.70)

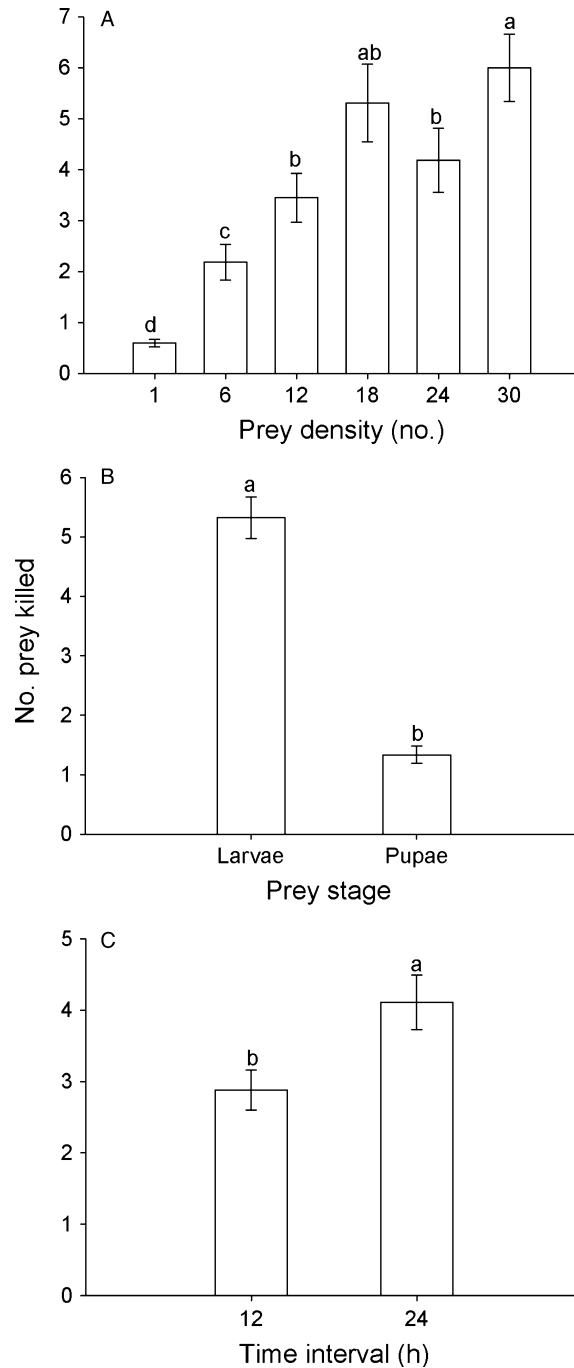


Figure 6 Mean ($\pm$ SE) number of *Alabama argillacea* prey killed by *Podisus nigrispinus* females at various (A) densities, (B) prey stages (larvae and pupae), and (C) time intervals. Means within a panel capped with different letters are significantly different (Student–Newman–Keuls test: $P < 0.05$).

found in no-choice conditions (Zanuncio et al., 2008). Larvae are variable in their defenses against natural enemies; larval defense mechanisms may be behavioral,

morphological, or physiological (Silva et al., 1996; Zanuncio et al., 2008). However, defense effectiveness varies according to the type of predator (Gentry & Dyer, 2002). Although predators are exposed to risk during the attack to the prey, they may minimize the prey's defensive actions (Edmunds, 1974). Often a predator is able to evaluate the potential risk provided by its prey and incidentally it may give up on an attack and abandon its prey (Braga et al., 2012). Larval movement may be a stimulating factor for the consumption of more prey (Pfannenstiel et al., 1995). The current study found higher attack capability by *P. nigrispinus* females to *A. argillacea* larvae compared to pupae. Perhaps the defensive response of *A. argillacea* larvae resulted in the predator's energetic wear, thus increasing its satiation level. Vacari et al. (2012) found functional response type II in *P. nigrispinus* preying on *Plutella xylostella* (L.) larvae and pupae. The defensive action by *P. xylostella* larvae encouraged *P. nigrispinus* predatory action (Vacari et al., 2013). We hypothesize that the same response may have occurred in *A. argillacea* because its pupae have low mobility and larvae have high mobility. In addition, the pupal casing of holometabolous insects may hinder the action of natural enemies (Oliveira et al., 2010), increasing the predator's unwillingness to consume the insect at this stage.

Bombyx mori L. larvae are not commonly preyed upon owing to the frequent movement of their head and other parts of their body (Lemos et al., 2005). In the case of *A. argillacea*, larvae were preyed upon more than pupae by *P. nigrispinus* females under all circumstances. The body flexibility of *A. argillacea* larvae may have made it more vulnerable to *P. nigrispinus*. After inserting the stylus, predatory stink bugs inject enzymes and substances that paralyze their prey in order to start extra-oral digestion. Moisture availability is essential for effective secretion of enzymes by the predator (Cohen, 1995). Therefore, the preference for larvae may also be due to greater availability of moisture in the larvae than in the pupae. Changes in the quantity and quality of food affect the predation capacity and thus a predator's life cycle, as individuals with a balanced diet reproduce more often and faster (Lemos et al., 2001). According to Khalequzzaman et al. (2007), *Phytoseiulus persimilis* Athias-Henriot preyed more on *Tetranychus urticae* Koch eggs, intermediate on the larva and nymph stages, and little on the adult stage. Predation by predatory bugs results in reduced feeding time of the prey (Zanuncio et al., 2005) and it is enough to change the handling time of the prey by the predator. *Podisus nigrispinus* handled *P. xylostella* pupae slower than larvae. Although females have invested more handling time in *A. argillacea* pupae, both in the 12- and 24-h periods, there was no significant difference as those found in larvae, in

both intervals. This absence of differences is attributed to the high behavioral variability revealed by the width of the confidence intervals and estimated by the models (CI 95%), especially for the females exposed to *A. argillacea* pupae.

Understanding the interaction between predators and their food resource helps to optimize biological control strategies aimed at defoliating pests. It also helps decision-making and the improvement of techniques to release *P. nigrispinus* in the field.

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References

- Azevedo FR & Ramalho FS (1999) Efeitos da temperatura e da defesa da presa no consumo pelo predador *Supputius cincticeps* (Stål) (Heteroptera: Pentatomidae). *Pesquisa Agropecuária Brasileira* 34: 165–171.
- Bale JS, van Lenteren JC & Bigler F (2008) Biological control and sustainable food production. *Philosophical Transactions of the Royal Society of London B* 363: 761–776.
- Bartlett MS (1937) Properties of sufficiency and statistical tests. *Proceedings of the Royal Society of London A* 160: 268–282.
- Braga RB, Corrêa AS, Della Lúcia TMC, Pereira AIA, Cruz I & Zanuncio JC (2012) Does the aggressiveness of the prey modify the attack behavior of the predator *Supputius cincticeps* (Stål) (Hemiptera, Pentatomidae)? *Revista Brasileira de Entomologia* 56: 244–248.
- Carneiro TR, Fernandes OA, Cruz I & Bueno RCOF (2010) Functional response of *Telenomus remus* Nixon (Hymenoptera, Scelionidae) to *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae) eggs: effect of female age. *Revista Brasileira de Entomologia* 54: 692–696.
- Cohen AC (1995) Extra-oral digestion in predaceous terrestrial Arthropoda. *Annual Review of Entomology* 40: 85–103.
- Costa LL, Funichello M & Busoli AC (2010) Parasitismo natural de ovos do curuquerê-do-algodoeiro por *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) em diferentes estágios fenológicos de variedades de algodoeiro em Ipameri, GO. *Bioscience Journal* 26: 281–286.
- De Clercq P, Mohaghegh J & Tirry L (2000) Effect of host plant on the functional response of the predator *Podisus nigrispinus* (Heteroptera: Pentatomidae). *Biological Control* 18: 65–70.
- Edmunds M (1974) *Defence in Animals: A Survey of Anti-Predator Defences*. Longman, Harlow, UK.

- Farhadi R, Allahyari H & Juliano SA (2010) Functional response of larval and adult stages of *Hippodamia variegata* (Coleoptera: Coccinellidae) to different densities of *Aphis fabae* (Hemiptera: Aphididae). *Environmental Entomology* 39: 1586–1592.
- Gentry GL & Dyer LA (2002) On the conditional nature of neotropical caterpillar defenses against their natural enemies. *Ecology* 83: 3108–3119.
- Haghani M, Fathipour Y, Talebi AA & Baniaméri V (2009) Estimating development rate and thermal requirements of *Hemiptarsenus zilahisebessi* (Hymenoptera: Eulophidae) parasitoid of *Liriomyza sativae* (Diptera: Agromyzidae) using linear and nonlinear models. *Polskie Pismo Entomologiczne* 78: 3–14.
- Hassanpour M, Mohaghegh J, Iranipour S, Nouri-Ganbalani G & Enkegaard A (2011) Functional response of *Chrysoperla carnea* (Neuroptera: Chrysopidae) to *Helicoverpa armigera* (Lepidoptera: Noctuidae): Effect of prey and predator stages. *Insect Science* 18: 217–224.
- Hassell MP (1978) *The Dynamics of Arthropod Predator-Prey Systems*. Princeton University Press, Princeton, NJ, USA.
- Hassell MP, Lawton JH & Beddington JR (1977) Sigmoid functional responses by invertebrate predators and parasitoids. *Journal of Animal Ecology* 46: 249–262.
- Holling CS (1959) The components of predation as revealed by a study of small mammal predation on the European pine sawfly. *Canadian Entomologist* 91: 293–320.
- Juliano SA (1993) Nonlinear curve fitting: predation and functional response curves. *Design and Analysis of Ecological Experiments* (ed. by SM Scheiner & J Gurevitch), pp. 159–182. Oxford University Press, New York, NY, USA.
- Khalequzzaman M, Mondal M, Haque MF & Karim MS (2007) Predatory efficacy of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) on the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *Journal of BioScience* 15: 127–132.
- Kontodimas DC, Eliopoulos PA, Stathas GJ & Economou LP (2004) Comparative temperature-dependent development of *Nephus includens* (Kirsch) and *Nephus bisignatus* (Boheman) (Coleoptera: Coccinellidae) preying on *Planococcus citri* (Risso) (Homoptera: Pseudococcidae): evaluation of a linear and various nonlinear models using specific criteria. *Environmental Entomology* 33: 1–11.
- Lemos WP, Medeiros RS, Ramalho FS & Zanuncio JC (2001) Effects of plant feeding on the development, survival and reproduction of *Podisus nigrispinus* (Heteroptera: Pentatomidae). *International Journal of Pest Management* 47: 89–93.
- Lemos WP, Zanuncio JC & Serrão JE (2005) Attack behavior of *Podisus rostralis* (Heteroptera: Pentatomidae) adults on caterpillars of *Bombyx mori* (Lepidoptera: Bombycidae). *Brazilian Archives of Biology and Technology* 48: 975–981.
- Madadi H, Parizi EM, Allahyari H & Enkegaard A (2011) Assessment of the biological control capability of *Hippodamia variegata* (Col.: Coccinellidae) using functional response experiments. *Journal of Pest Science* 84: 447–455.
- Malaquias JB, Ramalho FS, Souza JVS, Rodrigues KCV & Wanderley PA (2010) The influence of fennel feeding on development, survival, and reproduction in *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae). *Turkish Journal of Agriculture and Forestry* 34: 235–244.
- Malaquias JB, Ramalho FS, Omoto C, Godoy WA & Silveira RF (2014) Imidacloprid affects the functional response of predator *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) to strains of *Spodoptera frugiperda* (J.E. Smith) on Bt cotton. *Eco-toxicology* 23: 192–200.
- Malaquias JB, Omoto C, Ramalho FS, Wesley WAC & Silveira RF (2015) 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. *Journal of Pest Science* 88: 57–63.
- Medeiros RS, Ramalho FS, Serrão JE & Zanuncio JC (2004) Estimative of *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) development time with non linear models. *Neotropical Entomology* 33: 141–148.
- Michelotto MD, Crosariol Netto J, Grigolli JF, Busoli AC, Carrega WC et al. (2014) Effect of infestation of *Alabama argillacea* (Lepidoptera: Noctuidae) at different ages of cotton plants in yield. *American Journal of Plant Science* 5: 1441–1448.
- Mohaghegh J, De Clercq P & Tirry L (2001) Functional response of the predators *Podisus maculiventris* (Say) and *Podisus nigrispinus* (Dallas) (Het., Pentatomidae) to the beet armyworm, *Spodoptera exigua* (Hübner) (Lep., Noctuidae): effect of temperature. *Journal of Applied Entomology* 125: 131–134.
- Nascimento ARB, Ramalho FS, Azeredo TL, Fernandes FS, Nascimento Júnior JL et al. (2011) Feeding and life history of *Alabama argillacea* (Lepidoptera: Noctuidae) on cotton cultivars producing colored fibers. *Annals of the Entomological Society of America* 104: 613–619.
- Oliveira JEM, Bertoli SA, Miranda JE, Torres JB & Zanuncio JC (2008) Predação por *Podisus nigrispinus* (Heteroptera: Pentatomidae) sob efeito de densidades de *Alabama argillacea* (Lepidoptera: Noctuidae) e idades do algodoeiro. *Cientifica* 36: 1–9.
- Oliveira FQO, Batista JL, Malaquias JB, Almeida DM & Oliveira R (2010) Determination of the median lethal concentration (LC₅₀) of mycoinsecticides for the control of *Ceratitis capitata* (Diptera: Tephritidae). *Revista Colombiana de Entomologia* 36: 213–216.
- Pereira AIA, Ramalho FS, Rodrigues KCV, Malaquias JB, Souza JVS & Zanuncio JC (2010) Food extraction by the males of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) from cotton leafworm larvae. *Brazilian Archives of Biology and Technology* 53: 1027–1035.
- Pfannenstiel RS, Hunt RE & Yeagan KV (1995) Orientation of a hemipteran predator by feeding caterpillars. *Journal of Insect Behavior* 8: 1–9.
- Ramalho FS (1994) Cotton pest management: part 4. A Brazilian perspective. *Annual Review of Entomology* 39: 563–578.
- Ramalho FS, Mezzomo J, Lemos WP, Bandeira CM, Malaquias JB et al. (2008) Reproductive strategy of *Podisus nigrispinus* females under different feeding intervals. *Phytoparasitica* 36: 30–37.
- Ramalho FS, Azeredo TL, Fernandes FS, Nascimento Júnior JL, Malaquias JB et al. (2011) Food intake and utilization of *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae) fed on

- cotton cultivars with colored fibers. *Journal of Pest Science* 84: 199–205.
- Rogers D (1972) Random search and insect population models. *Journal of Animal Ecology* 41: 369–383.
- Rogers D (1975) A model for avoidance of superparasitism by solitary insect parasitoids. *Journal of Animal Ecology* 44: 623–628.
- Roy M, Brodeur J & Cloutier C (2002) Relationship between temperature and development rate of *Stethorus punctillum* (Coleoptera: Coccinellidae) and its prey *Tetranychus mcdanieli* (Acarina: Tetranychidae). *Environmental Entomology* 31: 177–187.
- SAS Institute (2006) SAS/STAT User's Guide. SAS Institute, Cary, NC, USA.
- Silva EN, Santos TM & Ramalho FS (1996) Desenvolvimento ninfal de *Supputius cincticeps* Stål (Hemiptera: Pentatomidae) alimentado com curuquerê do algodoeiro. *Anais da Sociedade Entomológica do Brasil* 25: 103–108.
- Silva GA, Picanço MC, Bacci L, Crespo ALB, Rosado JF & Guedes RNC (2011) Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. *Pest Management Science* 67: 913–920.
- Silva BR, Corrêa AS, Lúcia TMC, Pereira AIA, Cruz I & Zanuncio JC (2012) Does the aggressiveness of the prey modify the attack behavior of the predator *Supputius cincticeps* (Stål) (Hemiptera, Pentatomidae). *Revista Brasileira de Entomologia* 56: 244–248.
- Vacari AM, De Bortoli S & Torres JB (2012) Relationship between predation by *Podisus nigrispinus* and developmental phase and density of its prey, *Plutella xylostella*. *Entomologia Experimentalis et Applicata* 145: 30–37.
- Vacari AM, De Bortoli SA, Goulart RM, Volpe HXL, Otuka AK & Veiga ACP (2013) Comparison of eggs, larvae, and pupae of *Plutella xylostella* (Lepidoptera: Plutellidae) as prey for *Podisus nigrispinus* (Hemiptera: Pentatomidae). *Annals of the Entomological Society of America* 106: 235–242.
- Zamani AA, Talebi AA, Fathipour Y & Baniaméri V (2006) Temperature-dependent functional response of two aphid parasitoids, *Aphidius colemani* and *Aphidius matricariae* (Hymenoptera: Aphididae), on the cotton aphid. *Journal of Pest Science* 79: 183–188.
- Zanuncio JC, Beserra EB, Molina-Rugama AJ, Zanuncio TV, Pinon TBM & Maffia VP (2005) Reproduction and longevity of *Supputius cincticeps* (Het.: Pentatomidae) fed with larvae of *Zophobas confusa*, *Tenebrio molitor* (Col.: Tenebrionidae) or *Musca domestica* (Dip.: Muscidae). *Brazilian Archives of Biology and Technology* 48: 771–777.
- Zanuncio JC, Silva CAD, Lima ER, Pereira FF, Ramalho FS & Serrão JE (2008) Predation rate of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae with and without defense by *Podisus nigrispinus* (Heteroptera: Pentatomidae). *Brazilian Archives of Biology and Technology* 51: 121–125.