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ARTICULO DE INVESTIGACIÓN / RESEARCH ARTICLE

ECOLOGÍA

PREDATION OF *Brevicoryne brassicae* AND *Aphis craccivora* BY *Eriopis connexa* DEPENDING ON AVAILABILITY

Depredación de *Brevicoryne brassicae* y *Aphis craccivora* por *Eriopis connexa*, según disponibilidad

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ABSTRACT

Predator species under field conditions can face different and variable densities of prey species. This work evaluated the functional response of the neotropical lady beetle *Eriopis connexa* (Coleoptera: Coccinellidae) subjected to different densities of the aphids *Brevicoryne brassicae* and *Aphis craccivora* (Hemiptera: Aphididae). Thus, predation rates were analyzed of fourth-instar larvae and one-day old adults of the lady beetle preying upon the aphids at constant densities of 20, 40, 50, 60, and 70 aphids with 15 repetitions per density. The aphids were offered on 5 cm leaf discs of each plant host. The handling times and attack rates were 0.03 h^{-1} and 0.27 h^{-1} for larvae and 0.03 h^{-1} and 0.15 h^{-1} for adults fed *B. brassicae* and 0.59 h^{-1} and 0.35 h^{-1} for larvae and 0.70 h^{-1} and 0.95 h-1 for adults fed *A. craccivora*, respectively. Both larva and adult lady beetles increased predation rate as a function of prey density offered, with an estimated maximum number of prey consumed of 30.3 and 31.6 *B. brassicae* and 36.3 and 34.6 of *A. craccivora* by larva and adult lady beetles at the highest prey density, respectively. In conclusion, larvae and adults of *E. connexa* exhibited a type II functional response.

Keywords: aphidofagous, Biological Control, Entomology, Insecta.

RESUMEN

Las especies de depredadores en condiciones de campo pueden enfrentar densidades diferentes y variables de sus presas. Este trabajo evaluó la respuesta funcional de la mariquita neotropical *Eriopis connexa* (Coleoptera: Coccinellidae) sometida a diferentes densidades de los pulgones *Brevicoryne brassicae* y *Aphis craccivora* (Hemiptera: Aphididae). Se analizaron las tasas de depredación de larvas de cuarto estadio y adultos de un día de vida en las densidades constantes de 20, 40, 50, 60 y 70 pulgones con 15 repeticiones por densidad. Los pulgones se ofrecieron en discos de 5 cm de hojas de la planta huésped. Los tiempos de manejo y las tasas de ataque fueron 0.03 h^{-1} y 0.27 h^{-1} para larvas y 0.03 h^{-1} y 0.15 h^{-1} para adultos alimentados con *B. brassicae* y 0.59 h^{-1} y 0.35 h^{-1} para larvas y 0.70 h^{-1} y 0.95 h^{-1} para adultos alimentados con *A. craccivora*, respectivamente. Las larvas y las mariquitas adultas aumentaron la tasa de depredación en función de la densidad de presas ofrecidas, con un número máximo estimado de presas consumidas de 30.3 y 31.6 de *B. brassicae* y 36.3 y 34.6 de *A. craccivora* para larvas y mariquitas adultas a la mayor densidad de presas, respectivamente. En conclusión, las larvas y los adultos de *E. connexa* exhibieron una respuesta funcional del tipo II.

Palabras clave: afidofagas, Control Biológico. Entomología, Insecta.

INTRODUCTION

Predator Coccinellidae play a significant role in the biological control of pests in many agricultural crops (Biddinger et al., 2009; Obrycki, 2009; Weber and Lundgren, 2009), due to their ability to search out and consume pests (Vandenberg, 2002). The neotropical lady beetle, *Eriopis connexa* (Germar), consumes several types of preys, but prefers to attack aphids (Hagen, 1962; Oliveira *et al.,* 2004). *Eriopis connexa* has been studied as potential predator against pests in horticultural crops in the Neotropical region (Sarmento *et al.,* 2007; Duarte Gómez and Zenner de Polanía, 2009; Harterreiten-Souza *et al.,* 2012). In Brazil, this species is also commonly associated with pests of cultivated species, including key pests (Venzon *et al.,* 2009; Harterreiten-Souza *et al.,* 2012; Rodrigues *et al.,* 2013).

Predation is an important biotic factor that reduces pest insect populations in the natural environment and can reduce the use of insecticides for pest control (Wiedenmann and Smith, 1997; Sarmento *et al.,* 2007). In biological control, the relationship between prey density and consumption rate is known as functional response (Abrams and Ginzburg, 2000; Jeschke *et al.,* 2002). Functional response describes the behavior of the predator as a function of consumption at different densities, thus suggesting the maximum prey consumption, as well as the predator's potential in population control (Murdoch and Oaten, 1975). According to Solomon (1969), the increase in prey availability may lead the predator to increased consumption, up to a certain limit, because the opportunities to attack prey will increase over time.

Holling (1959) and Jervis and Kidd (1996) outline four fundamental types of functional response curves. Type I corresponds to the consumption of prey by the predator resulting in a linear increase. Type II occurs when the rate of predation decelerated reaching an asymptote. Type III involves a sigmoid relation also called S ascension, and Type IV establishes a dome-shaped curve. A predator that exhibits a type III functional response has greater potential to control prey population, because the proportion of prey attacked increases with increasing density (Fernandez-Arhex and Corley, 2003). However, type II functional response is most common for invertebrate predators (Van Lenteren and Bakker, 1976), because of satiation (e.g. predators) and ability to produce eggs (e.g. parasitoids), as well as restricted handling and attack time.

Thus, this study aimed to determine the functional response of the *E. connexa* lady beetles preying on the aphids *Brevicoryne brassicae* (Linnaeus, 1758) and *Aphis craccivora* Koch, 1854 at different densities.

MATERIAL AND METHODS

The experiments were conducted in the Entomology Laboratory from February 2017 to August 2017: Federal University of Alagoas (UFAL), Rio Largo, AL, Brazil.

Obtaining and Rearing of Predator and Preys

The lady beetles used in the experiment came from the rearing kept at the Insect Biological Control Laboratory of Universidade Federal Rural de Pernambuco -UFRPE. Adults and larvae were reared according to the methodology described in Rodrigues *et al.* (2013). The adults were raised in 500 mL plastic containers with lid opening covered with voile fabric to allow air circulation. Pieces of paper towel were placed inside the containers for oviposition substrate. The laid eggs were transferred daily to 500 mL plastic containers and held until hatching. Then, the larvae were transferred in 80 ml plastic containers at the density of two larvae per container. Inside the container, paper towel pieces of about 2 × 2 cm were placed as substrate for pupation. The insects were fed *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs, and in the adult phase were also offered a diet based on brewer's yeast and honey (1: 1).

The *Aphis craccivora* colony originated from lima beans (*Phaseolus lunatus* L.) (Fabaceae) grown in an experimental field of UFAL, in Rio Largo, AL, Brazil. These aphids were reared in cowpea plants (*Vigna unguiculata* (L.) Walp.) (Fabaceae), cultivar (Vita 7), susceptible to black aphid (Valente *et al.,* 2014). Cowpea plants were cultivated in 500 ml plastic cups containing soil and substrate in the ratio of 1:1 in cages protected with anti-aphid mesh (4.0 cm × 4.0 cm × 0.4 cm), kept in a greenhouse. Then, 15 days after planting, the plants were infested by apterous females placed with tweezers. At five-day intervals, the insects were transferred to new plants. This procedure was performed routinely to maintain breeding stock and use in studies.

The *Brevicoryne brassicae* colony was collected in cabbage plants of cv. Georgia, *Brassica oleracea* var. *acephala* D.C. (Brassicaceae), naturally infested in the field, in the UFAL Olericulture Sector, in Rio Largo, AL. These insects were transported to the laboratory and screened to remove parasitized individuals. They were then transferred to potted kale plants and kept in cages with anti-aphid mesh (1.0 m × 1.0 m × 0.5 m) in a greenhouse.

Functional Response of *Eriopis connexa* on the aphids *Brevicoryne brassicae* and *Aphis craccivora*

The predation rate of the lady beetle *E. connexa* on the aphids *B. brassicae* and *A. craccivora* was determined at different prey densities. This bioassay used fourth instar larvae and one-day-old adults of *E. connexa* and apterous adult aphids of *B. brassicae* and *A. craccivora*. Larvae and adult lady beetles used in the study were deprived of food for 24 hours to regulate the level of satiety. Six aphid densities were tested (20, 40, 50, 60, and 70) for both species with 15 replicates per density. The densities were established from the average rate of daily consumption obtained in preliminary tests.

The aphids were offered on 5.0 cm diameter leaf discs. The aphid *B. brassicae* was offered on cassava leaf discs, while the aphid *A. craccivora* was offered on cowpea leaf discs. Beforehand, the leaves were washed with water, neutral detergent, and 0.5 % sodium hypochlorite. When dry, the leaf discs were placed in 6.5 cm diameter Petri dishes lined with filter paper lightly moistened with distilled water and subsequently infested with the prey at respective densities. The transfer of prey was carried out with the aid of tweezers. After transfering the aphids to the plates, they were closed with PVC plastic film for 24 hours for the insects to settle. Fourth instar larvae along with adult lady beetles were individually transferred to Petri dishes according to the corresponding treatment, where they remained for 24 h. After that period, we recorded the number of live aphids remaining in each replicate and control without the presence of the predator, as well as the number of live lady beetle in each treatment.

In the first analysis step, the shape of the functional response curve was determined by logistic regression for the proportion of prey consumed as a function of the original prey densities per predator using Proc CATMOD in the SAS program, following the protocol described in Juliano (1993). Initially, the cubic model was tested because of its ability to capture all possible variations of the functional response curves. Then, the terms of the equation were reduced until their significance was obtained. The sign of the linear term of the equation generated from the proportion of prey consumed/killed by prey density was used to determine the type of functional response. When not significant, it indicates Type I functional response; a negative indicates Type II functional response; and a positive functional response indicates Type III. The parameters determined in the second step were handling time (Ht) and attack rate (a') of the functional response. These parameters were estimated by non-linear regression using the least squares method (PROC NLIN of the SAS) according to the methodology described by Juliano (1993) and compared by 95 % confidence interval.

RESULTS AND DISCUSSION

The mean prey consumption by fourth instar larvae and adults of *E. connexa* at different densities of adult *B. brassicae* and *A. craccivora* tended to stabilize at higher densities, resulting in a type II functional response curve. The estimated attack rate and handling time were, respectively, 0.026 and 0.0.34 h^{-1} for larvae and 0.029 h^{-1} and 0.60 h^{-1} for adults preying on *B. brassicae*; and 0.59 and 0.35 h^{-1} for larvae and 0.70 and 0.95 h^{-1} adults preying on *A. craccivora* (Tables 1 and 2). Both larvae and adult *E. connexa* increased the number of aphids preyed on with increased prey density until reaching asymptote. They consumed a maximum mean of 30.3 and 31.6 of *B. brassicae* and 36.3 and 34.6 of *A. craccivora*, of larvae and adults, respectively.

The Holling disc equation fit the results for the different stages of the *E. connexa* lady beetle on the different densities of both aphids (Table 1). When the proportion of prey consumed decreased for both larvae and adults of *E. connexa*, for both species of aphids, the number of preys consumed increased (Fig. 1 and 2). Pervez and Omkar (2005), studying the predation behavior of three lady beetle species *Cheilomenes sexmaculata* (Fabr.), *Propylea dissecta* (Mulsant) and *Coccinella*

Table 1 - Holling disc equation and functional response type of *Eriopis connexa* larvae and adults preying on the aphids *Brevicoryne brassicae* and *Aphis craccivora*.[1] Intercept. [2] Linear coefficient. [3] Quadratic coefficient. [4] Functional response

Treatments	Holling equation / Consumption ratio	Logistic regression coefficients			FR[4]
		I[1] (P)	L[2] (P)	Q[3] (P)	
Brevicoryne brassicae					
Larvae	$y = \frac{\exp\left(5.48 - 0.22x + 0.0035x^2\right)}{1 + \exp\left(5.48 - 0.22x + 0.0008x^2\right)}$	5.48 (<0.0001)	0.22 (<0.0001)	0.0035 (0.0070)	II
Adults	$y = \frac{\exp\left(4.91 - 0.25x + 0.0051x^2\right)}{1 + \exp\left(4.91 - 0.25x + 0.0051x^2\right)}$	4.91 (<0.0001)	0.254 (<0.0001)	0.0051 (<0.0001)	II
Aphis craccivora					
Larvae	$y = \frac{\exp\left(5.83 - 0.14x + 0.0008x^2\right)}{1 + \exp\left(5.83 - 0.14x + 0.0008x^2\right)}$	5.83 (<0.0001)	0.14 (<0.0001)	0.0008 (<0.0001)	II
Adults	$y = \frac{\exp\left(6.78 - 0.17x + 0.001x^2\right)}{1 + \exp\left(6.78 - 0.17x + 0.001x^2\right)}$	6.78 (<0.0001)	0.17 (<0.0001)	0.001 (<0.0001)	II

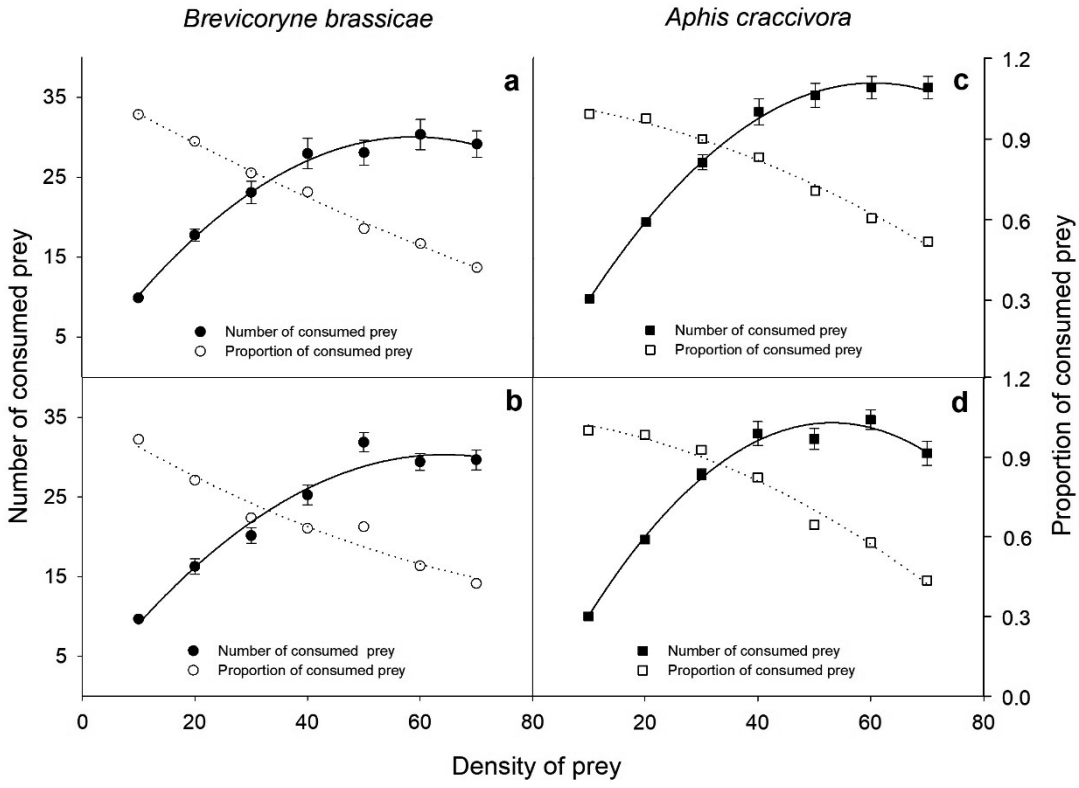

Figure 1 - (**a**) Functional response of fourth instar larvae and (**b**) adults of *Eriopis connexa* in different densities of the aphid *Brevicoryne brassicae*. (**c**) Functional response of fourth instar larvae and (**d**) adults of *Eriopis connexa* at different densities of aphid *Aphis craccivora*.

transversalis (Fabr.) (Coleoptera: Coccinellidae) on aphids *A. craccivora* and *Myzus persicae* (Sulzer), observed that all the lady beetles presented type II functional response for both preys. However, the handling time was quite varied among lady beetle species. The predators used in the Pervez and Omkar (2005) had longer handling time than the one found in this study. This demonstrates that each predator exhibits different predatory behavior with increased availability of prey; some are more voracious, especially those exhibiting shorter handling time and higher attack rates. (Mills, 1982; Ofuya and Akinbohungbe, 1988; Pervez, 2004). Handling time is an important variable of a predator's effectiveness and consumption rate, since it reflects the time that they take to kill, consume, and digest the prey (Veeravel and Baskaran, 1997).

This was clear in the present study, in which the attack and handling time were varied between the two aphid species offered. Both larvae and adults of *E. connexa* exhibited a less handling time and a lower attack rate praying on *B. brassicae*, than on *A. craccivora* (Table 1), which resulted in a higher predation on the latter species. This result was somewhat expected due to their different size, as well as other characteristics related to quality of prey, reflected in the prey species, as well as the age of the predator. Vieira *et al.,* (1997) found that fourth instar larvae of *Scymnus* (*Pullus*) *argentinicus* (Weise, 1906) (Coleoptera: Coccinellidae) used less handling time and preyed on older instars of *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae). This response of the predator can be attributed to the opportunity to encounter prey, which are greater depending on availability, time, and size. An increase in the availability of prey may lead to an increase in consumption, because prey is encountered more frequently, resulting in the consumption of an additional number of prey, even though the predator is already satiated (Garcia, 1990).

Type II functional response found for larvae and adults (Fig. 1) of *E. connexa* for both species of aphids, involves deceleration of the number of aphids consumed, due to

Table 2 – Handling time and attack rate of *Eriopis connexa* larvae and adults fed with the aphids *Brevicoryne brassicae* and *Aphis craccivora*.

Treatments	Handling Time (T_h, h^{-1}) (CI 95 %)	Attach rate (a', h^{-1}) (CI 95 %)
B. brassicae		
Larvae	0.026 (0.0250 – 0.0278)	0.34 (0.116 – 0.51)
Adults	0.029 (0.0282 – 0.0331)	0.60 (0.058 – 1.160)
A. craccivora		
Larvae	0.59 (0.529 – 0.653)	0.35 (0.1253 – 0.5917)
Adults	0.70 (0.6529 – 0.7627)	0.95 (0.3370 – 1.2245)

the increase in their availability, and is the most common type of predation response among coccinellids (Atlihan and Özgökçe, 2002; Pervez and Omkar, 2005; Britto *et al.,* 2009; Saleh *et al.,* 2010; Atlihan *et al.,* 2010). These results are also consistent with those obtained by Ofuya and Akingbohungbe (1988), in which the predator *Cheilomenes luneta* (Fabr.), feeding on the aphid *A. craccivora* presented a Type II functional response. Bortoli *et al.* (2014), evaluating the functional response of larvae and adults of lady beetle *Cryptolaemus montrouzieri* (Mulsant, 1850) on the cochineal *Planococcus citri*, also obtained type II functional response, for both phases.

Variable patterns of predation behavior are also a feature of *E. connexa*, which has various strategies when preying on different organisms. The lady beetle *C. sanguinea* (Linnaeus, 1763) presents type II response when preying on *Tetranychus evansi* (Thomas, 1878) (Acari: Tetranychidae). However, when the prey was the aphid *Macrosiphum euphorbiae* (Baker and Pritchard, 1960) (Hemiptera: Aphididae), it showed a type III functional response (Sarmento *et al.,* 2007), which characterizes a predation behavior change according to the type of available prey.

These results are relevant because the potential consumption of the studied aphid species by the lady beetle *E. connexa* is not known, although this lady beetle has been observed associated with infestations of these aphids (Rodrigues *et al.,* 2013; Costa *et al.,* 2018). Future research should focus on the biological performance of this lady beetle species feeding on these preys, as well as other pests common in bean and brassica crops. The susceptibility of *E. connexa* to insecticides recommended for use in these crops should be tested to conserve this lady beetle populations in crops. This species of lady beetle has shown high resistance to pyrethroid insecticides, which do not or minimally control aphids (Torres *et al.,* 2015; Costa *et al.,* 2018), but are commonly used to control leafhoppers in host cultures of these aphids. Therefore, maintaining *E. connexa* in these agroecosystems is important for apply integrated pest management. This could validate the predation of *B. brassicae* by this lady beetle in the field, since they are commonly associated with brassica crops.

CONCLUSION

Larvae and adults of *E. connexa* exhibit type II functional response when confined with different densities of aphids *B. brassicae* and *A. craccivora*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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