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Predation of *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae) on *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae)

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ABSTRACT: This research aimed to evaluate the consumption of *Hyadaphis foeniculi* by *Ceraeochrysa cubana* larvae, based on the densities and developmental stages of the prey, as well as the predator searching and handling time, regarding its instar and previous feeding knowledge. In order to evaluate the predation rate, larvae of first, second and third instars of *C. cubana* were used, which were fed with aphids from first and second instars, from third and fourth instar, and from different instars, at three densities. For searching time and handling evaluation, green lacewing larvae were submitted to different feeding regimens: second instar larvae fed at the first instar with *Anagasta kuehniella* eggs; second instar larvae, fed at the first instar with *H. foeniculi*; third instar larvae, fed at the first and second instars with *A. kuehniella* eggs; third instar larvae, fed at the first instar with the eggs of *A. kuehniella* and at the second instar with *H. foeniculi*; third instar larvae, fed at the first and second instars with *H. foeniculi*. It can be concluded that the daily *C. cubana* predation rate is influenced by *H. foeniculi* densities and instars, but the predator searching and handling times are not influenced by the diets provided to them in their previous stages.

Key words: aphids; consumption rate; fennel; green lacewing

Predação de *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae) sobre *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae)

RESUMO: Objetivou-se nesta pesquisa avaliar o consumo de *Hyadaphis foeniculi* por larvas de *Ceraeochrysa cubana* em função das densidades e fases da presa, bem como o tempo de busca e de manuseio do predador em função do ínstar e da sua experiência prévia de alimentação. Para avaliação da taxa de predação foram utilizadas larvas de 1º, 2º e 3º ínstares de *C. cubana*, as quais foram alimentadas com pulgões de 1º e 2º ínstares, pulgões de 3º e 4º ínstares e pulgões de diferentes ínstares, em três densidades. Para avaliação do tempo de busca e de manuseio, larvas do crisopídeo foram submetidas a diferentes regimes alimentares prévios: larvas de 2º ínstar, alimentadas no 1º ínstar com ovos de *Anagasta kuehniella*; larvas de 2º ínstar, alimentadas no 1º ínstar com *H. foeniculi*; larvas de 3º ínstar, alimentadas no 1º e 2º ínstares com ovos de *A. kuehniella*; larvas de 3º ínstar, alimentadas no 1º ínstar com ovos de *A. kuehniella* e, no 2º ínstar, com *H. foeniculi*; larvas de 3º ínstar, alimentadas no 1º e 2º ínstares com *H. foeniculi*. Pode-se concluir que a taxa de predação diária de *C. cubana* é influenciada pelas densidades e ínstares de *H. foeniculi*, mas os tempos de busca e de manuseio do predador não são influenciados pelas dietas ofertadas em seus estádios precedentes.

Palavras-chave: afídeos; taxa de consumo; erva-doce; crisopídeo

Introduction

Hyadaphis foeniculi (Passerini) (Hemiptera: Aphididae) is a cosmopolitan species and vector of at least 12 virus types, such as the Potyvirus and Yellow Spot Luteovirus. In Brazil, this aphid is the main pest of the fennel crop, *Foeniculum vulgare* (Mill.) (Malaquias et al., 2015). By continuous sap suction, those insects cause wilting and drying of both flowers and fruit alike (Ramalho et al., 2015), besides producing a sugary substance that favors development of *Capnodium* spp. fungus, which leads to the formation of fumagina, impairing plant respiration and decreasing photosynthetic area, thus contributing to its weakening (Leite et al., 2008; Baronio et al., 2015). In Paraíba state, a great producer of fennel, the fennel aphids usually reproduce at high temperatures seasons, forming insect colonies mainly on the flowers (Ramalho et al., 2015), but also being able to appear in seedlings and leaves.

In integrated pest management (IPM) programs, the biological control employment has become increasingly important, mainly when discussing integrated production towards sustainable agriculture (Sueldo et al., 2014). Predatory insects stand out among beneficial organisms responsible for the aphid population density regulation, with emphasis on those belonging to the Chrysopidae (Neuroptera) family, since they have suitable characteristics for biological control usage and are worldwide recognized for their occurrence in various agroecosystems (Tapajós et al., 2016).

In Brazil, one of the most studied chrysopidae species is the *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae), recommended for insect pest control (Oliveira et al., 2014; Oliveira et al., 2016). However, studies are still scarce regarding their predation behavior and potential in controlling some pest insects, such as the aphids.

According to Hernández et al. (2014), green lacewing are often effective agents in regulating the population density of various pest arthropods such as aphids. To this end, the relation between density and number of prey to be consumed is a fundamental aspect of the predator-prey dynamics, because depending on their density, prey can be used by natural enemies in different proportions and thus cause changes in the predator population. Another way of assessing the effectiveness of natural enemies in pest control is to determine the search and handling time, since the time spent by the predator to consume food is a factor that interferes with its search ability (Auad et al., 2002).

Predatory behavior of most green lacewings is still an underexplored theme; however, it is known that their larvae are active hunters, characterized by its fast movements and high search ability. During the prey capture process, according to Canard & Duelli (1984), after physical contact and identification of the prey, green lacewing larva stops moving immediately and acquires a characteristic posture, having the mouthparts wide open, parallel to the surface or slightly directed upwards, with the antennae and lip palms spread sideways. The capture of active prey is completed after a series of stereotyped predator behaviors: first) very slow approach; second) complete halt; third) sudden attack with rapid head advancement and mouthpiece closure, usually induced by the prey movement; fourth) head recoil following a rapid raising of the prey from the substrate, unless it is too heavy or strongly attaches to the surface. Immobile prey, such as eggs and pupae of arthropods and larvae of Coccidae and Diaspididae, are attacked differently since the green lacewing examines them thoroughly beforehand, tasting them with the tip of their mouthparts, and then puncturing their cuticle in several parts.

It is noteworthy that the search and handling time varies depending on the size of the predator and the prey, and the larva starvation state. For Bortoli et al. (2006), the type of supplied prey may influence predator efficiency, and the host in which that prey was bred may influence its quality, also affecting the predatory capacity of the green lacewing. Therefore, the objective of this research was to evaluate the consumption of *H. foeniculi* aphids by *C. cubana* larvae as a function of densities and phases of the prey, as well as to evaluate the predator search and handling time regarding the instar and its preview feeding experience.

Materials and Methods

The research was conducted at the Laboratory of Entomology (LEN) of the Center of Agricultural Sciences from Federal University of Paraíba – CCA/UFPB, Areia/PB, under temperature conditions of 25 ± 2 °C, relative humidity of 70 ± 10% and photoperiod of 12:12 h (Light:Dark).

Procurement of Aphids

The aphids were obtained from infestations in fennel plants kept in an experimental area of LEN/UFPB. Fennel seeds obtained from local commerce were sown part in the field and part in PVC pots (5 L), containing manure and sand, at 1:2 ratio, as substrate, aiming at the constant supply of aphids.

Predator breeding

Breeding of *C. cubana* began with procuring the specimens collected in the municipality of Matinhas/PB. Installation and establishment of the colony were performed by adapting to the methodology proposed by Moraes (1989).

C. cubana adults were kept in PVC (Polyvinyl Chloride) cylindrical cages (20.0 × 20.0 cm), sealed at their upper and lower ends with a nylon mesh and an aluminum lid respectively, internally lined with A4 paper for oviposition. Inside these containers five predator couples were placed, fed with a soft diet based on beer yeast and honey (1:1), which was added on a piece of bond paper (1.0 × 4.0 cm) and then placed inside the cage, being replaced every two days. Water was supplied every two days in wet cotton held in a lid of Ethylene Polyerephthalate (PET) (3.0 × 1.5 cm) inside the container, coinciding with the cleaning of the cages and the eggs removal.

After collection of the eggs, they were placed in ELISA plates (12.5 × 8.5 cm) containing 96 cells with 0.7 cm in diameter, coated with polyethylene film, having it been removed after the larvae hatching. When hatched, in order to prevent cannibalism, the larvae were individualized in transparent acrylic containers (3.5 × 2.0 cm), with a plastic lid containing small holes for their oxygenation, and fed with eggs of the flour moth *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) until the end of the larval stage. The eggs of this moth were from a mass rearing developed in the laboratory.

Consumption as function of prey density

To evaluate prey consumption, larvae of first, second and third instars of *C. cubana*. were used. The second and third instar larvae were fed at previous instars with *A. kuehniella* eggs. Immediately after the hatching of the first instar larvae and the ecdysis process of *C. cubana* second and third instar larvae, they were all individualized in the aforementioned transparent acrylic containers and kept without food for one hour. After this period, aphids of first and second instars, of third and fourth instars, and of different instars in three densities, were provided to the larvae, with equal distribution of the total number of prey for each prey instar (Table 1).

Densities were determined from the daily mean predation rate, considering one density below and one above the mean predation rate. The mean predation rate was determined from the number of aphids consumed in the predatory capacity test. After a 24 hours period, the number of aphids consumed by the green lacewing in each treatment was evaluated.

Table 1. Density of *Hyadaphis foeniculi* nymphs offered to *Ceraeochrysa cubana* larvae under laboratory conditions.

Densities	Number of offered aphids/green lacewing instar		
	First	Second	Third
1	10	35	60
2	20	45	70
3	30	55	80

Searching time and handling

In order to determine the search and handling time in each *C. cubana* instar, green lacewing larvae were individualized in the aforementioned transparent acrylic containers. The treatments and feeding (diet) of green lacewing larvae in the instars prior to their use in the trials were: Treatment 1: first instar larvae; Treatment 2: second instar larvae, fed at the first instar with *A. kuehniella* eggs; Treatment 3: second instar larvae, fed at the first instar with *H. foeniculi*; Treatment 4: third instar larvae, fed at first and second instars with *A. kuehniella* eggs; Treatment 5: third instar larvae, fed at the first instar with eggs of *A. kuehniella* and at the second instar with *H. foeniculi*; Treatment 6: third instar larvae, fed at the first and second instars with *H. foeniculi*.

For performing the trials, 40 nymphs of third and fourth instars of *H. foeniculi* were confined in Petri dishes (8.5 × 1.5 cm). Between 4 and 12 hours after the hatching or larvae ecdysis to second and third instars, all food was removed from the rearing units, leaving the green lacewing larvae without food for one hour. Subsequently, a *C. cubana* larva was released in the center of each dish and, by using a chronometer, the search and handling time were then determined, which were evaluated for each green lacewing instar, according to the feeding used previously at each phase. The searching time corresponded to the period in which the predator was exposed to the prey until its capture. The period in which he remained in contact with the prey consuming it corresponded to the handling time.

Statistical analysis

For the consumption analysis as a function of the prey densities, a completely randomized design was used, in a 3 × 3 × 3 factorial scheme, represented by the three *C. cubana* instars, three *H. foeniculi*-based diets (aphids of first and second instars, of third and fourth instars, and of different instars) and three prey densities. Each treatment consisted of 10 replicates. For analysis of the searching and handling time, the statistical design used was completely randomized, with 6 treatments and 10 replicates. Data were transformed into log (×) and subsequently submitted to the analysis of variance, with the treatment means compared by the Tukey test at 5% significance , using a generalized linear model. Analyzes were performed employing the SAS 9.3® software (SAS Institute, 2011).

Results and Discussion

Prey consumption regarding density

When evaluating the mean daily predation rate of *H. foeniculi* aphids by *C. cubana* larvae, it was found that the density factor and the different prey feeding regimes were significant ($p = 0.01$), as well as the interactions aphid × density and green lacewing × aphid × density, pointing out that triple interaction influences the green lacewing consumption (Table 2).

A growing consumption of aphids from different phases as a function of green lacewing instars was verified, except at density 3, observing that in the aphids diet with first and

Table 2. Summary of analysis of variance for the mean predation rate of *Hyadaphis foeniculi* aphids by *Ceraeochrysa cubana* larvae in different diets and densities.

Variation source	DF	MS
Green lacewing [z]	2	54858.96**
Aphid[y]	2	5128.60**
Green lacewing × aphid	4	334.02**
Density[w]	2	3280.01**
Green lacewing × density	4	565.88**
Aphid × density	4	101.83**
Green lacewing × aphid × density	8	474.19**
Error	243	29.73

[z] Green lacewing: first, second e third instars.
[y] Aphid: offered at first and second instars, third and fourth instars and different instars.
[w] Densities of aphids offered to *C. cubana*: 10, 20 and 30 aphids for the first instar; 35, 45 and 55 aphids for the second instar; and 60, 70 and 80 aphids to the third predator instar.
** Significant for the *F* Test ($p = 0.05$).

second instars, the predator consumption of second instar larvae was similar to that of first instar, since they did not differ statistically, and lower than the obtained consumption at density 2, for the same diet (Table 3). This result may infer that the predator reached the satiety level, ingesting the amount of food adequate for the nutritional needs and development of the second instar, even at a lower prey density.

At density 1, the mean daily predation rate of aphids during the first instar of *C. cubana* was not influenced by the different prey diets, fact that did not occur in the other green lacewing instars for density 1 and for densities 2 and 3. (Table 3). At all densities (1, 2, 3), there was a higher consumption of first and second instar aphids for the three stages of green lacewing, except for the ones from second instar of density 3, where it was observed a lower consumption of first and second aphids, in relation to the diet with aphids from different instars, being statistically different. The higher consumption factor of first and second instar aphids can be explained by the prey size in relation to the predator, being necessary more aphids to reach satiety in this case, since, when in this phase, aphids offer few defense capabilities, increasing the effectiveness chances of the predator in his predation ability. Yet, larvae fed with third and fourth instars aphids had lower mean predation rate (Table 3).

According to Carvalho & Souza (2009), small prey with less mobility and that have a thin cuticle easily pierced by the mouthparts, are suitable for feeding green lacewing larvae, reinforcing the higher consumption of first and second instar aphids and lower consumption of third and fourth instars aphids by *C. cubana* larvae.

Regarding the influence of prey density on green lacewing predation, similar results were observed by Santos et al. (2005) when studying the mean daily predation rate of aphid *Aphis gossypii* (Glover) (Hemiptera: Aphididae) at different densities by *Chrysoperla externa* (Hagen) larvae (Neuroptera: Chrysopidae); they found that the daily intake by the first instar larvae predator was lower at density 1 (10 aphids), in comparison to densities 2 and 3 (15 and 20 aphids, respectively). Also during the second instar of the green lacewing, satiety density 2 (40 aphids) was observed, which used approximately the amount provided in this study, showing that the number of prey consumed was sufficient to meet the nutritional requirements for its complete daily development. In a similar way, Fonseca et al. (2000) identified similar results when studying the mean predation rate of *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) by *C. externa* larvae in the three instars, at different densities.

An increase in prey availability may lead the predator to increase consumption as opportunities of meeting prey will be greater. However, once satiated, the predator is unable to continue consuming prey, maintaining its nutritional needs for approximately 24 hours. The results found in this research are the basis for studies on predatory behavior and functional response analysis of *C. cubana* with *H. foeniculi* prey. Functional response studies are important for assessing predatory efficiency of natural enemies (Bahar et al., 2013; Flores et al., 2013).

Table 3. Mean aphid predation rate (±EP) *Hyadaphis foeniculi* at different densities and diets by *Ceraeochrysa cubana* larvae.

D	GI	Aphid-based diets		
		First e second AI	Third e fourth AI	DI
1	First	8.1 ± 0.43 Ca	3.20 ± 0.29 Ca	4.2 ± 0.29 Ca
	Second	32.3 ± 0.75 Ba	11.5 ± 0.89 Bc	22.5 ± 2.29 Bb
	Third	56.9 ± 1.02 Aa	32.4 ± 4.15 Ab	51.7 ± 2.22 Aa
2	First	14.6 ± 0.76 Ca	7.5 ± 0.60 Bb	9.2 ± 0.95 Cb
	Second	39.7 ± 1.33 Ba	12.8 ± 1.55 Bc	21.7 ± 1.70 Bb
	Third	69.8 ± 0.20 Aa	52.5 ± 3.21 Ac	64.0 ± 2.74 Ab
3	First	24.6 ± 1.00 Ba	10.9 ± 1.04 Cb	15.0 ± 0.86 Cb
	Second	22.5 ± 1.44 Bb	20.9 ± 1.41 Bb	35.1 ± 2.00 Ba
	Third	79.6 ± 0.31 Aa	60.6 ± 2.88 Ab	60.8 ± 2.04 Ab

Means followed by the same uppercase letter in the column and lowercase in the row do not differ statistically from each other by the Tukey test at 5% probability. D = aphid densities; GI = green lacewing instars; AI = aphid instars; DI = different aphid instars.

Searching time and handling

Searching time for *C. cubana* first instar larvae fed with *H. foeniculi* was longer when compared to the other instars, differing significantly from the second instar, whose first instar larvae were fed with *H. foeniculi*, and the third instar, regardless of the previous feeding; however, it did not differ statistically from the second instar, whose larvae from the previous stage fed upon *A. kuehniella* eggs (Table 4).

For Nunes et al. (2017), the smaller prey size in relation to the predator and its fast movement may make the first instar larvae need more time to find the prey. Under field conditions, prey finding by the predator can be facilitated by infestation and gregarious aphid behavior in plants. Therefore, due to the generalist habit, *C. cubana* larvae end up finding available food.

In the second instar of *C. cubana*, there was no statistical difference in searching time as a function of feeding in the preceding larva stage (Table 4), as observed by Auad et al. (2002), who studied the searching time for *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) in *Uroleucon ambrosiae* (Thomas) (Hemiptera: Aphididae). The same result was found in the third instar of the predator, where no significant difference was observed in the searching time regarding the food used in previous instars. However, larvae of this instar were faster in the prey searching than those from other phases, regardless of the feeding with which the larvae were previously fed.

Table 4. Searching and handling time (‘min:”seg) of *Ceraeochrysa cubana* larvae, at the three instars, fed with *Hyadaphis foeniculi.*

Predator instar and previous feeding used	Searching	Handling
First	00:04’:03’’ a	00:13’:14’’ a
Second *Ak*	00:01’:22’’ ab	00:13’:38’’ a
Second *Hf*	00:01’:01’’ b	00:16’:15’’ a
Third *Ak* + *Ak*	00:00’:52’’ b	00:11’:45’’ a
Third *Ak* + *Hf*	00:00’:34’’ b	00:05’:44’’ a
Third *Hf* + *Hf*	00:00’:44’’ b	00:07’:22’ ’a

Means followed by the same letter in the column do not differ statistically from each other by the Tukey test at 5% probability. Ak = *A. kuehniella*; Hf = *H. foeniculi*. Data transformed in log(x).

Maia et al. (2004), when studying the predatory capacity and biological aspects of *C. externa* fed with *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae), also found a longer searching time for the predator first instar larvae when compared to what was found at the second and third larvae instars. This fact was also observed by Fonseca et al. (2000) when studying the functional response of *C. externa* fed with *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). Macedo et al. (2010), studying the biological and behavioral aspects of *C. externa* in cotton, observed no significant difference in the time of aphid *A. gossypii* search for first, second and third instars larvae of *C. externa*. According to Albuquerque (2009), green lacewing larvae prey usually exhibit gregarious behavior. As such, the time required for the first encounter with the prey tends to be longer, especially for first instar larvae, probably due to their low mobility when compared to larvae from subsequent instars.

Handling time of *H. foeniculi* nymphs by *C. cubana* larvae did not present significantly difference between predator instars, as well as in relation to previous diets (Table 4). Nunes et al. (2017), in a study on biological aspects and behavior of *C. cubana* predation on *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), found that the mean time taken by the chrysopidae to attack and consume prey decreased according to its development.

Although no significant differences in handling time were detected, the time spent on manipulating and consuming prey by third instar *C. cubana* larvae was shorter when compared to other instars, especially when fed with *H. foeniculi* (Table 4), which may be related to the fact that the predator body volume undergoes significant increases at each instar, leading to a faster suction of the prey hemolymph, with consequent reduction in handling time, if compared to first instar larvae green lacewing, which have lower feeding activity (Hassanpour et al., 2015).

The handling time of *C. externa* larvae fed by *U. ambrosiae*, determined by Auad et al. (2002), under similar conditions, was higher to that found in this study for all green lacewing instars, which may indicate that the *C. cubana* species can be considered an excellent biological controlling agent of *H. foeniculi*.

Conclusions

The mean daily predation rate of *C. cubana* is influenced by both densities and instars of *H. foeniculi*.

Predator searching and handling times are not influenced by the offered diets in their previous phases, showing that the used feeding does not affect the predator efficiency.

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