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Biological aspects and predation behavior of *Ceraeochrysa cubana* against *Spodoptera frugiperda*

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ABSTRACT

Mortality of neonate larvae and eggs of *Spodoptera frugiperda* has an important contribution to the management of this pest. This work aimed to determine the development, quantify the consumption, the time of search and handling of eggs of *S. frugiperda* by *Cereaochrysa cubana*. The duration of development and the survival of *C. cubana* were determined using eggs of the alternative prey *Anagasta kuehniella*; eggs, newborn caterpillars and combined feeding of egg + newborn caterpillars of *S. frugiperda*. The daily and total consumption, the search and handling time were also evaluated, comparing eggs and caterpillars of *S. frugiperda* as prey. The duration of the larvae to adult phase of *C. cubana* was prolonged when the food was newborn caterpillars or eggs + newborn caterpillars of *S. frugiperda* (35.7 and 34.6 days) when compared to eggs of *A. kuehniella* and *S. frugiperda* (25.5 and 25.9 days). The predator's larvae showed a shorter larval and adult period when fed with eggs in relation to caterpillars. Larval viability of *C. cubana* was lower when fed with eggs + newborn caterpillars. Higher consumption and shorter search and handling time on newborn caterpillars of *S. frugiperda*, compared to prey eggs, are carried out by larvae of *C. cubana* during the third instar.

Key words: biology; chrysopodes; fall armyworm

Aspectos biológicos e comportamento da predação de Ceraeochrysa cubana sobre Spodoptera frugiperda

RESUMO

A mortalidade de ovos e larvas neonatas de *Spodoptera frugiperda* possui importante contribuição para o manejo desta praga. Este trabalho objetivou determinar o desenvolvimento, quantificar o consumo, o tempo de busca e de manipulação de ovos de *S. frugiperda* por *Cereaochrysa cubana*. A duração do desenvolvimento e a sobrevivência de *C. cubana* foram determinadas empregando-se ovos da presa alternativa *Anagasta kuehniella*; ovos, lagartas neonatas e alimentação combinada de ovos + lagartas neonatas de *S. frugiperda*. Também foram avaliados os consumos diário e total, o tempo de busca e de manipulação, comparando-se ovos e lagartas de *S. frugiperda* como presa. A duração da fase de larva a adulto de *C. cubana* foi prolongada quando o alimento foi lagartas neonatas ou ovos + lagartas neonatas de *S. frugiperda* (35,7 e 34,6 dias) comparado a ovos de *A. kuehniella* e *S. frugiperda* (25,5 e 25,9 dias). Larvas do predador apresentam menor período de larva-adulto quando alimentados com ovos em relação a lagartas. A viabilidade larval de *C. cubana* foi menor quando alimentadas com ovos + lagartas neonatas. O maior consumo e menor tempo de busca e de manipulação sobre lagartas neonatas de *S. frugiperda*, em comparação aos ovos da presa, são realizados por larvas de *C. cubana* durante o terceiro instar.

Palavras-chave: biologia; crisopídeos; lagarta-do-cartucho

Introduction

The main plague affecting corn in Brazil is the *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Bueno et al., 2010; Machado et al., 2014), which can feed on other species of plants to stay in the agroecosystems, among them crops of great economic importance and alternative hosts (Barros et al., 2010; Boregas et al., 2013). Its attack occurs from the neonate phase to the last larval instar, promoting both quantitative and qualitative damages to the cultures.

The main forms of control of *S. frugiperda* are associated to the use of resistant varieties, transgenic plants and, mainly, synthetic insecticides (Pinto et al., 2013; Souza et al., 2013). Frequent and indiscriminate use of insecticides to control *S. frugiperda* is one of the main obstacles to an environmentally sustainable production and generates concerns about the possibility of favoring the occurrence of insecticide-resistant populations (Farias et al., 2014). Sueldo et al. (2014) reported that the use of biological control as a method to reduce pest infestation in crops is essential, mainly because it is a safe and environmentally correct method, and is essential for the establishment of integrated pest management (IPM) programs.

Chrysopidae species are found in different crops and have potential in the control of several arthropod pests such as aphids, cochineals, eggs and larvae of Lepidoptera, among others (Costa et al., 2012; Hernández, 2014; Tapajós et al., 2016). The search for a species with a high predation rate and presenting satisfactory survival characteristics when fed with the target insect pest is one of the essential steps for the proper decision to choose a natural enemy and its inclusion in integrated management programs.

The species *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae) has been found in different crops of economic importance, such as corn (Resende et al., 2014) and recommended for the control of insect pests (Alcantra et al. Oliveira et al., 2014). Therefore, this work aimed to determine the development of *C. cubana* when fed with *S. frugiperda*, as well as the consumption, attack and handling of prey.

Material and Methods

This research was developed in the Laboratory of Entomology (LEN), Department of Plant Science and Environmental Sciences of the Center of Agricultural Sciences of the Federal University of Paraíba - DFCA/CCA/UFPB, Areia-PB. The experiment was conducted under laboratory conditions: mean temperature of 25 ± 2° C, relative humidity of 70 ± 10% and photophase of 12 hours.

Adults of *C. cubana* originated from the rearing at LEN/CCA/UFPB, kept in cylindrical PVC cages (20 cm high x 20 cm in diameter) coated with sulphite paper and closed on top with voile fabric, both serving as oviposition substrate, while the lower opening was supported on zinc adapted to the structure of the cage. The adults' diet consisted of a diet based on brewer's yeast + honey (1: 1) and water, replaced every two days.

Development

C. cubana eggs were collected and packed in plastic Elisa® plates and covered with polyethylene film, until hatching. Subsequently, the larvae were individualized in plastic pots with 3.5 cm high and 2.5 cm in diameter.

In order to evaluate the development of *C. Cubana*, a completely randomized design was used with four treatments containing 20 replicates each, using one predator larvae per replicate. The larvae of *C. cubana* were submitted to feeding with *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) (T1) – standard, *S. frugiperda* eggs (T2), newborn caterpillar of *S. frugiperda* (first instar with up to two days of age) (T3) and with the combination of eggs + newborn caterpillars of *S. frugiperda* (T4). The prey *S. frugiperda* was fed with artificial diet adapted to the proposal by Nalin (1991).

The larvae of *C. cubana* were exposed to food and the larval period was determined by recording the duration of each instar daily. The larval viability was also determined as the ratio of total pupae obtained without defects by the initial number of larvae in each treatment.

Eggs of *A. kuehniella* and *S. frugiperda* were supplied to the predator in transparent plastic pots (3.5 cm high and 2.5 cm in diameter). In order to evaluate the development of *C. cubana,* the scales present in the layers of *S. frugiperda* eggs were partially unstructured. Newborn caterpillars were offered to the predator in Petri dishes covered with plastic film and eggs + newborn caterpillars were supplied alternately to avoid cannibalism, a common behavior of this species.

The larval and pupal period were determined, as well as the viability of both through the relation between the total number of pupae obtained and the number of adults emerged alive and without apparent defects. After the emergency, the insects were separated for evaluation of sexual ratio.

For the development of larval and pupal phases, data were submitted to analysis of variance and the means of treatments were compared by the Tukey test at 5% of probability, while the values of viability and sexual ratio were analyzed by the chi-square test (X^2) and compared by the Tukey test at 5% of probability.

Behavior of predation

Eggs, supplied with the natural oviposition structure (T1), and *S. frugiperda* newborn caterpillars (T2) were used to evaluate the parameters related to predation. Both were offered in Petri dishes (9.0 cm in diameter x 1.5 cm in height) to C. *cubana* larvae. The prey consumption was determined for the three instars of the larval phase of the predator. Daily, the amount of prey consumed was counted until the change of instar and, subsequently, the total consumption was calculated. The search and handling times (attack and consumption) were determined by putting the prey in the center of the plaque and releasing a *C. cubana* larva that had been without food for up to 24 hours. Time monitored with the aid of a stopwatch.

A completely randomized statistical design was used, and 10 replicates were used for each treatment. In the predatory behavior, the treatments were arranged in a 2 x 3 factorial scheme (food x instars of the predator), and the means of treatments were compared by the F test and Tukey test at 5% probability.

The data of each experiment were submitted to analysis of variance and the software used for the statistical analysis was Sisvar 5.3 Build 77 (Ferreira, 2011). The evaluation of the difference between viability and sexual ratio was performed using Proc GLIMMIX from SAS (SAS Institute, 2006).

Results and Discussion

Larvae of *C. cubana* of first and second instars showed longer duration of development when fed with *S. frugiperda* (F = 150.9; *P* <0.001; G.L. = 3), whereas third instar larvae confined with eggs + newborn caterpillars of *S. frugiperda* showed development of 10.9 days (Table 1). This result was similar to that found by Oliveira et al. (2016), when *C. cubana* was fed with eggs of *Sitotroga cereallella* (Olivier) (Lepidoptera: Gelechiidae). These authors also verified longer durations for the larval stages of this predator when it was fed with nymphs of *Aleurochanthus woglumi* Ashby (Hemiptera: Aleyrodidade).

Feeding on *S. frugiperda* eggs provided a larval development similar to the prey *A. kuehniella*, usually used for rearing chrysopodes in all larval stages of *C. cubana*. Also, the larval phase was statistically similar when *C. cubana* consumed eggs + newborn caterpillars or, only, caterpillars of *S. frugiperda* (Table 1). The duration of the pre-pupal + pupal period of *C. cubana* was lower in the treatments eggs of *A. kuehniella* and eggs + newborn caterpillars of *S. frugiperda*. In contrast, the period from larva to adulthood was shorter only in the treatments containing eggs of both lepidopteran species. Adults obtained in each treatment did not present differences in the sexual ratio (Table 2).

The nutritional adequacy of *S. frugiperda* eggs to the requirements of *C. cubana* in pupation probably is not deleterious to the percentage of pupae obtained, but to the time to reach this phase, and it needs food supplementation. Other chrysopodes had a shorter duration of the pre-pupal + pupal phase when exposed to feeding with lepidopteran eggs and caterpillars (Almeida et al., 2009).

The viability of the first instar of *C. cubana* showed no differences between treatments (P> 0.50). The second and third instars presented less viability in the treatment of eggs + newborn caterpillars of *S. frugiperda* (Table 3). On the other hand, greater viability of the larval stage was found in the treatments with lepidopteran eggs (P <0.01). Pre-pupal viability was lower when *C. cubana* larvae were fed with *S. frugiperda* newborn caterpillars, and higher when they consumed lepidopteran eggs (P <0.01). The highest viability of the pupal phase was 100%, observed in the treatment of newborn eggs of *S. frugiperda* (P <0.05) (Table 3).

The highest values of viability of the larval phase are close to those verified by other authors when providing *S. cerealella* eggs to chrysopodes (Pessoa et al., 2010), with values ranging from 95 to 100%, which emphasizes the nutritional quality of lepidopteran eggs in the feeding of *C. cubana* larvae. Thus, the survival of the predatory insect, especially when it is generalist, can vary according to the prey species and the variation of prey consumed. Oliveira et al. (2016) evidenced non-adequacy of eggs of citrus blackfly for larval survival of *C. cubana*, while Bonani et al. evidenced that aphids *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) or mealybugs *Planococcus citri* (Risso) (Hemistera: Pseudococcidae) supplied to larvae of *Chrysoperla externa* (Hagen) (Neuroptera: Crysopidae) in laboratory do not constitute adequate food to this predator. Similarly, Pedro Neto et al. (2008) observed that feeding *C. externa* with the acarus *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae) and/or *P. citri* showed satisfactory survival results only when eggs of *A. kuehniella* are added to the diet. Also, first-instar larvae of *C. externa* and *Ceraeochrysa*

Table 2. Duration (± SE) of periods pre- pupal + pupal, larva to adult and sexual ratio (sr) of *Ceraeochrysa cubana*, reared with *Spodoptera frugiperda* in laboratory. Temperature of 25 ± 2° C, RH of 70 ± 10% and 12-hour photophase.

Treatment[1]	Pre-pupal + pupal	Larva to adult	sr[2]
Anagasta kuehniella eggs	14.9 ± 0.62 b	25.9 ± 0.64 b	0.60
Spodoptera frugiperda eggs	15.2 ± 0.50 ab	25.5 ± 0.87 b	0.60
S. frugiperda newborn catterpillars	16.7 ± 0.40 a	35.7 ± 0.69 a	0.50
S. frugiperda eggs + newborn caterpillars	14.9 ± 0.33 b	34.6 ± 0.60 a	0.73
Coefficient of variation (%)	13.03	9.71	

[1] Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.[2] Statistical difference by chi-square test (χ^2 = 1.72; G.L. = 3; P > 0.05).

Table 1. Duration (± SE) of each instar and larval phase of *Ceraeochrysa cubana* bred with different preys in laboratory. Temperature of 25 ± 2° C, RH of 70 ± 10% and 12-hour photophase.

Treatment[1]	Duration of instars and phase (days)			
	First	Second	Third	Larval
Anagasta kuehniella eggs	3.2 ± 0.09 c	3.2 ± 0.10 c	4.0 ± 0.19 c	10.5 ± 0.19 b
Spodoptera frugiperda eggs	3.1 ± 0.05 c	3.2 ± 0.11 c	3.9 ± 0.13 c	10.3 ± 0.24 b
S. frugiperda newborn catterpillars	6.3 ± 0.24 a	6.7 ± 0.23 a	6.5 ± 0.22 b	19.0 ± 0.43 a
S. frugiperda eggs + newborn caterpillars	4.2 ± 0.27 b	4.7 ± 0.19 b	10.9 ± 0.62 a	19.7 ± 0.51 a
Coefficient of variation (%)	20.76	16.5	20.34	11.53

[1] Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.

Table 3. Viability of each instar, in the larval (LP), pre-pupal (PP) and pupal (P) phases of *Ceraeochrysa cubana*, reared with *Spodoptera frugiperda* in laboratory. Temperature of 25 ± 2° C, RH of 70 ± 10% and 12-hour photophase.

Treatment[1]	Viability (%)					
	First	Second	Third	LP	PP	P
Anagasta kuehniella eggs	95.0 a	100.0 a	100.0 a	98.3 a	84.2 a	52.6 c
Spodoptera frugiperda eggs	95.0 a	100.0 a	100.0 a	98.3 a	89.5 a	52.7 c
S. frugiperda newborn catterpillars	85.0 a	88.2 b	81.3 b	84.8 b	46.2 c	66.7 b
S. frugiperda eggs + newborn caterpillars	90.0 a	77.8 c	78.6 c	82.1 b	63.0 b	100.0 a
χ^2 values	1.7[ns]	8.4*	7.8*	12.2*	12.3*	8.0*

[1] [ns]Non-significant differences and * Significant differences by the test χ^2 (G.L. = 3). Values followed by the same lowercase letter do not present statistical differences by the Tukey test at 5% of probability.

everes (Banks) (Neuroptera: Chrysopidae) reared with the mealybugs *Ferrisia virgata* Cockerell and *Pseudococcus jackbeardsleyi* Gimpel & Miller (Hemiptera: Pseudococcidae) were unsuccessful, preying exclusively third instar nymphs or females adults of mealybugs, which present the body covered by cerosity. However, second-instar nymphs of mealybugs allowed the development of larvae of all the ages of chrysopodes (Tapajós et al., 2016). In relation to chrysopodes pupation, when a prey is not a nutritionally adequate food, the combination of different preys may imply a higher survival of the species, as described by Santa-Cecília et al. (1997). In the field of corn production, for example, the predator can find other food resources in the host plant of *S. frugiperda*, such as aphids, leafhopper nymphs and even pollen, which may favor its development.

C. cubana presented satisfactory results in relation to another chrysopodis, *S. frugiperda*. Comparing the biological development of *C. externa* consuming *S. frugiperda* eggs and newborn caterpillars of different ages, in laboratory conditions similar to that of this research, Tavares et al. (2011) showed values of larval viability of 45 to 70% and pupal viability between 20 and 45%.

The daily consumption of *S. frugiperda* by *C. cubana* larvae increased according to the larval stage of the predator, being higher on the newborn caterpillars of the pest (Figure 1A). Third-instar larvae consumed around 47.4 eggs and 63.5 newborn caterpillars. With the development of the larva, its size increased and there was need of a larger amount of food to reach the pupal stage (Oliveira et al., 2014). The highest total predation of *C. cubana* occurred according to the instar of the predator, being statistically higher for third-instar larvae consuming a larger number of newborn caterpillars compared to *S. frugiperda* eggs at this stage (Figure 1B).

Because the eggs of *S. frugiperda* are arranged in layers and present scales, they have a defense mechanism that can hinder the predator's access, thus, it can favor a lower consumption. Protection structures are reported as factors that influence the predation of chrysopodes. The consumption capacity of *C. externa* was influenced by the presence of the web involving the mites *Tetranychus evansi* (Baker & Pritchard) (Acari: Tetranychidae) (Venzon et al., 2009), as well as by the secretion of pulverulent wax that covers the bodies of the mealybugs *P. citri*, *F. virgata* and *P. jackbeardsleyi* (Bonani et al., 2009; Tapajós et al., 2016).

The higher consumption of *C. cubana* on newborn caterpillars in relation to eggs may be associated with the low nutritive content of the prey, as well as to the stimulus to predation due to movement. Nutritive content is cited in other chrysopodes predators of lepidopterans (Almeida et al., 2009; Tavares et al., 2011).

First-instar larvae of *C. cubana* showed longer time to reach the eggs of *S. frugiperda* than second-instar larvae to reach caterpillars; and those of third instar had similar search time. The comparative search behavior between the instars showed third-instar larvae are more agile on the eggs and, together with those of first instar, on caterpillars (Table 4).

The average time *C. cubana* takes to attack and consume the prey has decreased according to its development. First-

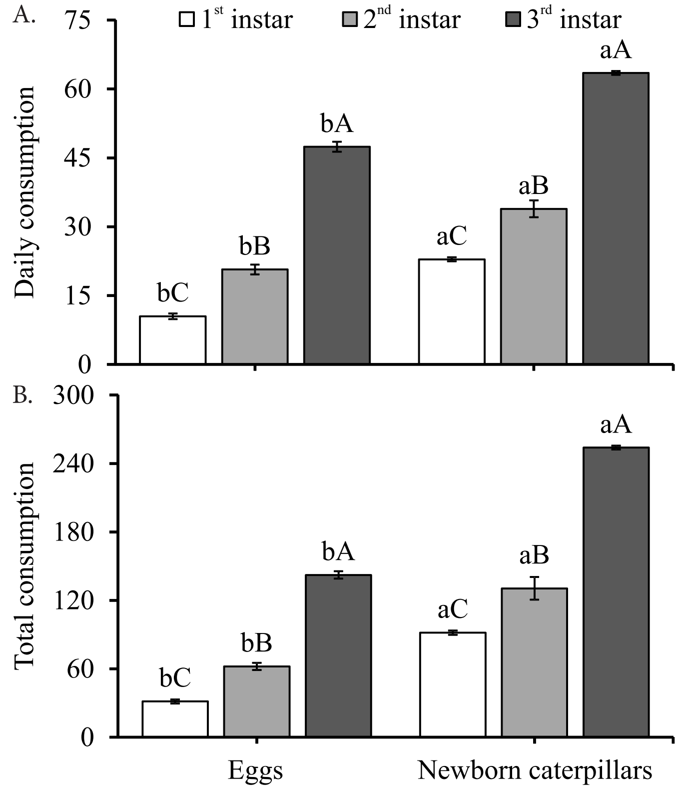

Figure 1. Average daily number of eggs and newborn caterpillars of *Spodoptera frugiperda* consumed by *Ceraeochrysa cubana* in their different larval stages. Columns followed by the same lowercase letters do not differ between treatments and upper case letters between the instars, respectively, by the F and Tukey test at 5% of probability.

Table 4. Mean time of search and handling (min ± SE) of instars of *Ceraeochrysa cubana* preying eggs or larvae of *Spodoptera frugiperda* in the laboratory. Temperature of 25 ± 2° C, RH of 70 ± 10% and 12-hour photophase.

Treatment[1]	Search time		
	First	Second	Third
Spodoptera frugiperda eggs	8.02 ± 0.86 aA	6.08 ± 0.55 bA	4.00 ± 1.25 aB
S. frugiperda newborn caterpillars	5.01 ± 0.38 bB	10.38 ± 0.59 aA	5.09 ± 0.55 aB
CV (%)		14.85	
		Handling time	
Spodoptera frugiperda eggs	30.88 ± 1.77 bA	10.09 ± 0.39 bB	6.15 ± 1.19 aC
S. frugiperda newborn caterpillars	49.16 ± 1.11 aA	17.23 ± 0.89 aB	7.54 ± 0.29 aC
CV (%)		7.17	

[1] Means followed by the same lowercase letter in the column and upper case in the row do not differ statistically from each other by the F and Tukey test, respectively, at 5% of probability.

instar larvae presented longer handling time in predating caterpillars and longer time in relation to the other instars in both types of prey (Table 4). Second-instar larvae also spent more time consuming *S. frugiperda* caterpillars and third-instar caterpillars had a mean time of consumption that was statistically similar in both treatments and lower in relation to the other instars.

The presence of the scales and hair covering the layers of eggs may be a factor that hindered the predation of *C. cubana*, especially in the first instar. Ecole et al. (2002) observed that the predation of *C. externa* on eggs and larvae of *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) was inhibited due to the conformation of eggs and the difficulty of tearing the mine where the larvae are located. Since third-instar larvae of *C. cubana*, are larger and more aggressive than the younger ones, the former can overcome the prey protections. The different predation behavior of the third-instar of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) on eggs and caterpillars of *Helicoverpa armigera* (Hübner) was verified by Hassanpour et al. (2010), confirming the most effective attack and consumption activity of these larvae. The defense movements of the newborn caterpillars are features that can affect the performance of the predators, as observed by Zanuncio et al. (2008) for the functional response of the predator *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae), a fact that may have occurred with small larvae of *C. cubana*.

Handling activity of *S. frugiperda* by *C. cubana* involves attack, extra-oral digestion, ingestion of the liquefied contents of the prey, and finally placing the prey (totally or partially consumed) on the back. *C. cubana* larvae under the confinement conditions of this study only ceased handling after ingesting completely the attacked prey. Ferreira (2008) evidenced an average manipulation time of 26.28 minutes for first-instar larvae of *C. cubana* consuming nymphs of third and fourth instars of *Aphis gossypii* Glover (Hemiptera: Aphididae). The size and voracity of third-instar larvae of *C. cubana* certainly was a preponderant factor for the shortest handling time. First-instar larvae of chrysopodes present lower food activity (Hassanpour et al., 2015).

Conclusions

Larvae of *C. cubana* presented lower larval period and higher survival when reared with *S. frugiperda* eggs in relation to predation on caterpillars of this pest.

Third-instar larvae of *C. cubana* showed higher consumption and shorter search and handling time against *S. frugiperda* newborn caterpillars compared to prey eggs.

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