

## THERMAL REQUIREMENTS OF *Ceraeochrysa cubana* (Neuroptera: Chrysopidae) EMBRYONIC PERIOD SUBMITTED TO ARTIFICIAL DIETS AT ADULT STAGE

*EXIGÊNCIAS TÉRMICAS DO PERÍODO EMBRIONÁRIO DE Ceraeochrysa cubana (Neuroptera: Chrysopidae) SUBMETIDA A DIETAS ARTIFICIAIS NA FASE ADULTA*

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**ABSTRACT:** Green lacewings can be mass reared to market their eggs. The ideal temperature is a main factor for egg storage or scheduling of insect production. This study aimed to evaluate the influence of *Ceraeochrysa cubana* adult feeding on incubation period and feasibility of eggs storage under different temperatures. Adults were submitted to four artificial diets: honey + brewer's yeast (standard diet), molasses + brewer's yeast, honey + bee pollen and molasses + bee pollen. Incubation period was recorded keeping eggs at six different temperatures: 21, 23, 25, 27, 29 and 31 °C. requirements. We determined the duration of the incubation period and egg feasibility of *C. cubana*. In addition, we estimated the base temperature (*T<sub>b</sub>*) and thermal threshold (*K*) for the green lacewing incubation. Regardless of diet, incubation period decreases according to increased temperature, which is indicated between 21 and 29 °C for better development and egg feasibility. Adult feeding influences thermal constant, however, does not influence base temperature of the eggs, which varies from 15 to 18 °C. Our results can serve as a basis for the choice of adults' diet and temperature for the storage of eggs during the mass rearing of *C. cubana*.

**KEYWORDS:** Egg storage. Green lacewings. Mass rearing. Temperature.

### INTRODUCTION

The high infestation intensities of arthropod-pests that affect crops of economic importance generate considerable damage to agricultural production. Biological control is one of the activity reduction tactics of these individuals, a basic pillar for the implementation of integrated pest management (IPM) (NARANJO et al., 2015).

Among the natural control agents of insect-pests, predator insects known as green lacewings (Neuroptera: Chrysopidae) are studied (LIRA; BATISTA, 2006; BEZERRA et al., 2009; PAPPAS et al., 2011; TAVARES et al., 2011; OLIVEIRA et al., 2016). In Brazil, *Chrysoperla* and *Ceraeochrysa* genus stands out. *Ceraeochrysa cubana* species (Hagen, 1861) (Neuroptera: Chrysopidae), known commonly as trash-carrying chrysopids, is found in different crops, as maize and tomato (RESENDE et al., 2014; MOURA et al 2015), and is an important predator of many pests in its larval stage (ALCANTRA et al., 2008; OLIVEIRA et al., 2014; NUNES et al., 2017). In addition, its ease rearing outside natural environment is a factor considered as essential. In adult stage lacewings feed on nectar,

pollen and honeydew (PARRA et al., 2002; MORGADO et al., 2014). These foods can be replaced by artificial diets consisted of energy and protein sources in its formulation.

Less expensive diets optimize mass rearing of insect predators, which aim at producing on a scale that allows its release for pest control in field (VAN LENTEREN, 2012). In large-scale rearing, lacewing feeding can influence longevity of adults and egg production, as well in period and feasibility of incubation period (VENZON et al., 2006; OLIVEIRA et al., 2009). The temperature is another importance factor for development stage of lacewings (CANARD; PRINCIPI, 1984), mainly aiming at egg storage for marketing or scheduling for the management of insect production (LÓPEZ-ARROYO et al., 2000; AMARAL et al., 2013; MORGADO et al., 2014). Thus, this study aimed to evaluate the influence of *C. cubana* adult feeding on incubation period and feasibility of eggs storage under different temperature, characterizing base temperature and thermal constant in order to determine a temperature range in which eggs can be submitted.

## MATERIAL AND METHODS

This study was conducted in the Laboratory of Entomology of the Federal University of Paraíba. *C. cubana* adults kept under rearing in laboratory were used, with an average temperature of  $25 \pm 2$  °C, relative humidity of  $75 \pm 10\%$  and photoperiod of 12 hours. Adults were reared in PVC cylindrical cages, locked at the bottom with zinc lid and on top with white voile fabric, and white paper internally adhered to cages. One artificial diet consisted of honey and brewer's yeast was provided for lacewings in equal proportions (1:1) arranged in a rectangular plastic material (2.5 x 0.5 cm), and distilled water provided on hydrophilic cotton stored in polyethylene lids.

Green lacewing eggs were collected and placed for hatching in plastic plates (ELISA®). After hatching, larvae were fed with *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) eggs for obtaining adults. A total of 10 couples of *C. cubana* were stored in small transparent plastic cages (one couple per cage). Four different diets were used: Honey + brewer's yeast; Molasses + brewer's yeast; Honey + bee pollen; Molasses + bee pollen. We used honey associated with brewer's yeast as standard diet. The bee pollen in the artificial diets was based on Loru et al. (2014), and molasses in due to the fact that this component has facility of acquisition and possibility of utilization as food attractants for lacewings adults under field conditions (KUNKEL; COTTRELL, 2014).

Sugar cane molasses was purchased at supermarket. Honey was derived from the production of Beekeeping sector, Campus II of UFPB; brewer's yeast and bee pollen from herbal medicines store. Diets were provided in adapted transparent plastic material (polyethylene), with 0.5 x 1.5 cm dimensions. Water was placed in hydrophilic cotton stored in lids for plastic pots (1.0 cm height x 1.5 cm diameter) with the aid of spray bottle. The different diets provided for the *C. cubana* couples in each temperature were replaced every two days.

During feeding of adults with diets cited above, eggs produced were collected and separated into ELISA plates and subjected to different temperatures: 21, 23, 25, 27, 29 and 31 °C, in air-conditioned chambers of Biological Oxygen Demand (B.O.D.) type, with relative humidity of  $70 \pm 10\%$  and of 12L:12D photoperiod. So, the following parameters were evaluated: duration of incubation period (days) and egg feasibility (hatchability) (%). These parameters were used to

determine the duration of the incubation period of *C. cubana* and ratio between hatching number of larvae without apparent defects. Days of development, development speed, base temperature (*T<sub>b</sub>*) and thermal threshold (*K*) were also estimated for incubation period. We used four replications for each diet with 10 samples/replication, totaling 40 plots/temperature.

The statistical design adopted for data analysis related to the effect of each separate temperature on incubation period in each treatment was completely randomized. Data were submitted to analysis of variance (ANOVA) and treatment means were compared by Tukey test at 5% probability. To evaluate the feasibility of incubation period, a 4 x 6 factorial scheme was adopted (diets x temperatures). Interaction data between different diets with temperatures and duration of embryonic period were submitted to polynomial regression testing, and *T<sub>b</sub>* and *K* were estimated from the hyperbola equation (HADDAD et al., 1999). The statistical software used for research was *Sisvar*® 5.3 Build 77 (FERREIRA, 2011).

## RESULTS

At 21 °C temperature was observed that the shorter duration of *C. cubana* incubation period occurred when the green lacewing was fed with honey + bee pollen. There was no statistical difference in duration of *C. cubana* incubation period between diets, when eggs were submitted to 23, 25, 27 and 29 °C temperatures. By rising temperature to 31 °C, hatching was faster when adults were mainly fed with standard diet and honey + bee pollen, approximately for three days (Table 1).

We observed *T<sub>b</sub>* values for *C. cubana* incubation period ranging from 14.4 to 15.7 °C, and thermal constant, calculated according to speed of development curve, between 48.3 and 54.9 degrees-day. According to confidence intervals, diets provide no effect on base temperature, but influence thermal constant. The honey + brewer's yeast and molasses + brewer's yeast diets are approaching the interval found in molasses + bee pollen diet and are far from the obtained interval for honey + bee pollen diet (Table 2).

From variance analyses, significant interaction was observed between treatments and temperature under days of development ( $p > 0.01$ ) and development speed ( $p < 0.01$ ) of *C. cubana* incubation period, where all treatments were significantly linearly at all temperatures (Figure 1).

**Table 1.** Duration of *C. cubana* incubation period submitted to different diets at adult stage and with eggs kept under different temperature in B.O.D air-conditioned chambers, RH of 70 ± 10% and 12L:12D photoperiod

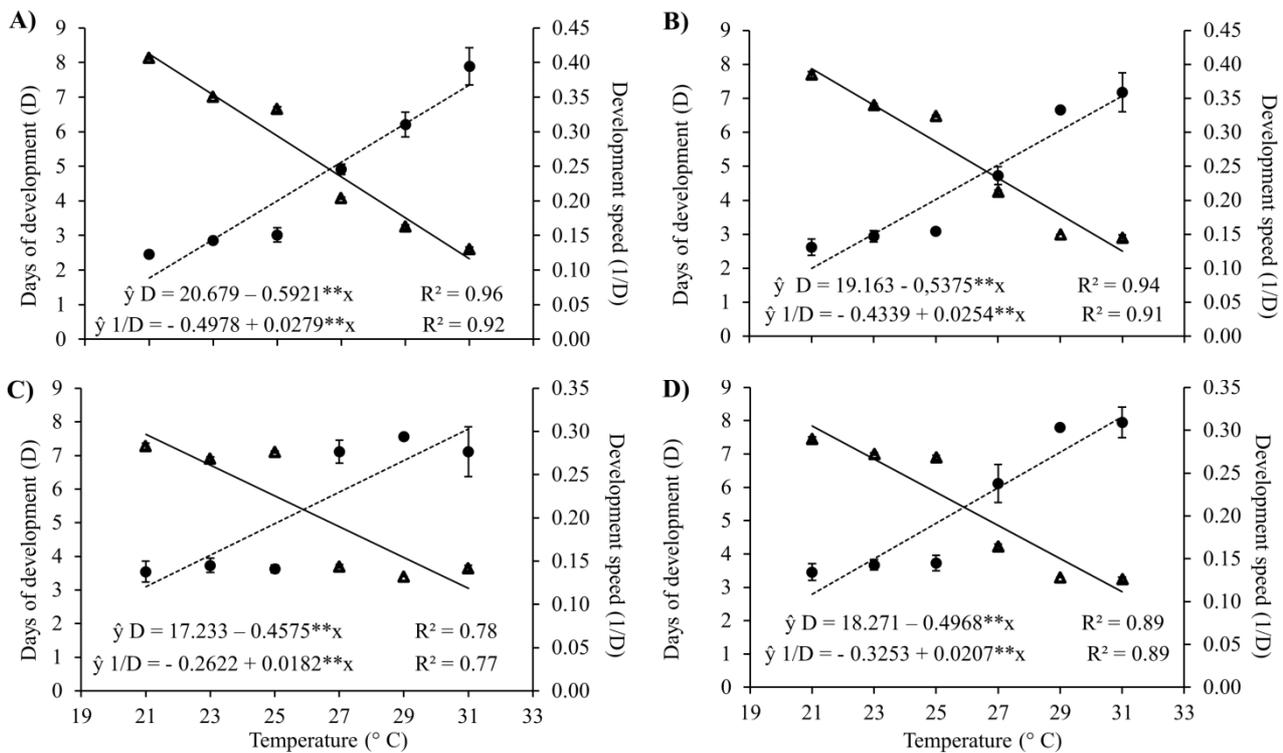
Temperature	Diets				CV(%)
	H + BY	M + BY	H + BP	M + BP	
21 °C	8.1 ± 0.07 a	7.7 ± 0.42 ab	7.3 ± 0.25 c	7.5 ± 0.21 ab	6.29
23 °C	7.0 ± 0.04 a	6.8 ± 0.16 a	6.9 ± 0.09 a	7.0 ± 0.08 a	2.69
25 °C	6.7 ± 0.23 a	6.5 ± 0.05 a	7.1 ± 0.20 a	6.9 ± 0.11 a	4.31
27 °C	4.1 ± 0.05 a	4.3 ± 0.17 a	3.7 ± 0.32 a	4.2 ± 0.17 a	8.65
29 °C	3.3 ± 0.18 a	3.0 ± 0.00 a	3.4 ± 0.00 a	3.3 ± 0.07 a	3.95
31 °C	2.6 ± 0.23 b	2.9 ± 0.33 b	3.7 ± 0.21 ab	3.3 ± 0.13 a	14.45

Means ± standard error followed by same lowercase letter in line do not statistically differ by Tukey test (p<0.05). H+BY = honey + brewer's yeast; M+BY = molasses + brewer's yeast; H+BP = honey + bee pollen; M+BP = molasses + bee pollen.

**Table 2.** Linear regression equations between temperatures (21, 23, 25, 27, 29, 31 °C) and speed of development (1/D) to determine base temperature (Tb) and thermal constant (K) in development of *C. cubana* incubation period (adults submitted to different diets)

Diets	a*	b**	Pr>F	R <sup>2</sup>	Tb (°C) (CI <sup>1</sup> )	K (Degree-days) (CI)
H+BY	-0.4978	0.0279	<0.0001	0.9245	17.8 (13.2-23.7)	35.8 (32.4-41.0)
M+BY	-0.4339	0.0254	<0.0001	0.9116	17.1 (12.3-23.5)	39.3 (34.8-45.0)
H+BP	-0.2622	0.0182	<0.0001	0.7731	14.4 (7.9-23.2)	54.9 (47.5-68.8)
M+BP	-0.3253	0.0207	<0.0001	0.8873	15.7 (9.9-23.6)	48.3 (42.2-58.1)

<sup>1</sup>Confidence intervals (95%); Coefficient \*linear and \*\*angular from line obtained by regression equation for 1/D; Tb= -a/b; K = 1/b. H+BY = honey + brewer's yeast; M+BY = molasses + brewer's yeast; H+BP = honey + bee pollen; M+BP = molasses + bee pollen



**Figure 1.** Regression curves adjusted for influence of different diets for *C. cubana* on days of development (—) and development speed (-----) of its incubation period (eggs subjected to different temperatures). A) H+BY= honey + brewer's yeast; B) M+BY = molasses + brewer's yeast; C) H+BP = honey + bee pollen; D) M+BP = molasses + bee pollen.

The feasibility of *C. cubana* incubation period fed with different diets statistically differ in relation to 27 °C temperature, by having 100% of viable eggs in molasses + brewer's yeast diet and 75% in molasses + bee pollen diet. By comparing the feasibility of the eggs within each temperature according to food provided for the adults of

lacewing, it was found that only in diets containing bee pollen has temperature influence on incubation period (Table 3). When diet with molasses + bee pollen diet was provided to *C. cubana* adults we observed lower value of egg feasibility at 27 °C temperature (Table 3).

**Table 3.** Feasibility (%) of *C. cubana* incubation period submitted to different diets with eggs kept under different temperatures in B.O.D. chambers, RH of 70 ± 10% and 12L:12D photoperiod

Temperature	Diets			
	H + BY	M + BY	H + BP	M + BP
21 °C	92.5 ± 2.5 Aa	87.5 ± 2.5 Aa	90.0 ± 5.8 Aa	90.0 ± 4.1 ABa
23 °C	95.0 ± 2.9 Aa	95.0 ± 2.9 Aa	85.0 ± 5.0 Aa	87.5 ± 6.3 ABa
25 °C	92.5 ± 4.8 Aa	97.5 ± 2.5 Aa	97.5 ± 2.5 Aa	97.5 ± 2.5 Aa
27 °C	92.5 ± 4.8 Aab	100.0 ± 0.0 Aa	82.5 ± 6.3 Abc	75.0 ± 2.9 Cc
29 °C	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa	95.0 ± 5.0 Aa	90.0 ± 4.1 ABa
31 °C	92.5 ± 4.8 Aa	100.0 ± 0.0 Aa	97.5 ± 2.5 Aa	90.0 ± 4.1 ABa

Mean ± standard errors followed by same capital letters in the column and lowercase in line are statistically differ by Tukey test ( $p < 0.05$ ); CV (%) = 7.35. H+BY = honey + brewer's yeast; M+BY = molasses + brewer's yeast; H+BP = honey + bee pollen; M+BP = molasses + bee pollen

## DISCUSSION

As temperature was enhanced from 21 °C to 31 °C, duration and development speed of incubation period tended to be decreasing and increasing, respectively. The hatching time of larvae was higher in standard treatment at lower temperatures and decreased with raising temperature. The same occurred in molasses + brewer's yeast treatment; however, values of stage duration are lower at mild temperatures and increase compared to standard diet only when temperature exceeds 29 °C. Rather than diets, differences between duration of incubation period can be different among chrysopid species. Decreased duration of incubation period due to increased temperature are common to lacewings, as observed for *C. externa* (ALBUQUERQUE et al., 1994; PESSOA et al., 2009), *Chrysoperla raimundoi* Freitas & Penny, 2001 (FREITAS; PENNY, 2001), and *Chrysoperla carnea* (Stephens, 1836) (NADEEM et al., 2012) at temperatures ranging between 20 and 35 °C.

At assessment of *C. externa* embryonic period duration fed on *Alabama argillacea* (Hübner, 1818) (Lepidoptera: Noctuidae) at larval stage and on standard diet at adulthood, Silva et al. (2002) showed decrease as temperature enhanced from 15 °C to 30 °C. Decrease of 32% in duration of *C. raimundoi* embryonic period was observed by feeding adults with standard diet and eggs subjected

to a temperature of 31 °C (LAVAGNINI et al., 2009). The values observed by these authors are equal to those observed in this study, when *C. cubana* is fed on the same diet and diet based on molasses and brewer's yeast.

Our results indicated that there is relationship among diets for adults to thermal requirements of *C. cubana* incubation period, and some values are according to other studies with other chrysopid species. The thermal constant observed in honey + bee pollen diet is similar to that reported for *C. raimundoi* (LAVAGNINI et al., 2009). Bezerra et al. (2012) found values of  $T_b = 11.08$  and  $K = 51.39$  for *Chrysoperla genanigra* Freitas, 2003 (Neuroptera: Chrysopidae) incubation period.

It is observed in literature recommendations and optimal temperature ranges for feasibility of lacewing eggs, between 20 and 25 °C (PESSOA et al., 2009; BEZERRA et al., 2012). Diet based on molasses and brewer's yeast provided 100% of feasibility at temperatures between 27 °C and 31 °C. This temperature range is according to that evidenced for the feasibility of *Dichochrysa prasina* (Burmeister, 1839) (Neuroptera: Chrysopidae) eggs (PAPPAS et al., 2008).

The relationship between temperature and embryonic stage of lacewings is important for its development and survival and is fundamental for storage of eggs and stock control in mass rearing (LÓPEZ-ARROYO et al., 2000). The results of

Amaral et al. (2013) confirmed that the development of *C. externa* embryo is lower at temperatures of 12 °C and should be removed from the storage according to age at which egg was subjected to storage. Our results can be important to supervision of the *C. cubana* egg storage at mass rearing conditions.

## CONCLUSIONS

Regardless of the diet used for *C. cubana*, the duration of embryonic period decreases with increased temperature at which eggs are subjected.

Temperatures between 21 °C and 29 °C are the most suitable for the storage of eggs, and the interaction between diets based on molasses +

brewer's yeast with temperature of 27 °C promotes lower *C. cubana* egg hatchability.

The thermal constant of incubation period is influenced by the food provided for adults, in contrast, base temperature is not influenced, ranging from 15 to 18 °C.

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**RESUMO:** Os crisopídeos podem ser criados em grande escala para comercialização dos ovos. A temperatura ideal é um fator decisivo para o armazenamento de ovos ou escalonamento da produção do inseto. Essa pesquisa objetivou avaliar a influência da alimentação dos adultos de *Ceraeochrysa cubana* no período de incubação e na viabilidade dos ovos armazenados em diferentes temperaturas. Os adultos foram submetidos a quatro dietas artificiais: mel + lêvedo de cerveja (padrão), melado + lêvedo de cerveja, mel + pólen apícola e melado + pólen apícola. O período de incubação foi registrado mantendo-se os ovos em seis temperaturas diferentes: 21, 23, 25, 27, 29 e 31 °C. Determinou-se a duração do período de incubação e a viabilidade dos ovos de *C. cubana*. Além disso, estimaram-se a temperatura base ( $T_b$ ) e a constante térmica ( $K$ ) para o período de incubação do crisopídeo. Independente da dieta, o período de incubação decresce de acordo com o aumento da temperatura, a qual tem faixa indicada para melhor desenvolvimento e viabilidade dos ovos entre 21 e 29 °C. A alimentação dos adultos influencia na constante térmica, mas não influencia na temperatura base dos ovos, que varia de 15 a 18 °C. Nossos resultados podem servir de base para a escolha da dieta dos adultos e da temperatura para armazenamento dos ovos durante a criação massal de *C. cubana*.

**PALAVRAS-CHAVE:** Criação massal. Crisopídeos. Estocagem de ovos. Temperatura.

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