Biology and thermal requirements of *Chrysoperla genanigra* (Neuroptera: Chrysopidae) reared on *Sitotroga cerealella* (Lepidoptera: Gelechiidae) eggs

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**ABSTRACT**

*Chrysoperla genanigra* Freitas is a common green lacewing associated with melon pests in the Northeastern Brazil. All life stages of this recently described species were studied under a range of constant temperature conditions (17, 21, 25, 29, 33, 35 and 37 °C), a photoperiod of 12 h:12 h (L:D) and 70 ± 10% relative humidity. Adults of *C. genanigra* were fed on a diet consisting of a 1:1 (v/v) mixture of brewer's yeast and honey, while larvae were provided with eggs of *Sitotroga cerealella* (Olivier) *ad libitum*. The duration of preimaginal development of the species was inversely proportional to temperature and ranged from approximately 63 days at 17 °C to 15 days at 35 °C. The percentage of adult emergence varied from 6.7% at 17 °C to 76.7% at 25 °C, although no larvae were able to complete development at 37 °C. The lower thermal threshold for total preimaginal development was approximately 10.8 °C and the thermal requirement was 336.7 degree-days. Egg production, along with the longevity of both males and females, were significantly affected by temperature. It is concluded that the best temperature for rearing *C. genanigra* is 25 °C, with the lowest preimaginal mortality and the highest egg production (992.7 eggs/female).

**Keywords:** Agricultural entomology, Biological control, Development, Green lacewing, Mass rearing, Temperature
1. Introduction

Larvae of green lacewings are generalists and are well known for their predatory efficiency, their ability to seek out food and their high survival rate in agroecosystems (Canard and Principi, 1984). Four members of the genus Chrysoperla (Steinmann, 1964) are represented among the numerous species of lacewings found in Brazil, and they were reported to exhibit a wide range of predatory attributes (Freitas, 2003; King and Nordlund, 1992). Over the last few decades, there has been increasing interest in the mass rearing of exotic species (Albuquerque et al., 1994; Tauber et al., 2000). In this context, the recently described species Chrysoperla genanigra Freitas from the Northeastern region of Brazil (Freitas, 2003) has been shown to be associated with the melon agroecosystem in that area and may have utility in pest control management systems for melon (Bezerra et al., 2010). C. genanigra is very similar to the other Chrysoperla species in Brazil, and can be easily recognized due to its gena color, which is pale with a black inferior margin, while the other species have red gena. Nevertheless, species confirmation should always be done by examining the male genitalia (Freitas and Penny, 2001; Freitas, 2003). Melon production is one of the most technically developed activities in Northeastern Brazil, especially in the states of Rio Grande do Norte and Ceará. These two are responsible for more than 80% of Brazilian melon production (325,000 ton in 2009), where about 90% is exported (US$ 89 million in 2010), with the EU as the main destination. The melon productive chain generates around 28,000 direct jobs and 84,000 indirect jobs in Northeastern Brazil (IBRAF, 2011; IBGE, 2009). All this production chain is threatened around 28,000 direct jobs and 84,000 indirect jobs in Northeastern Brazil (IBRAF, 2011; IBGE, 2009).

2. Material and methods

2.1. Stock colony of C. genanigra

Adult specimens of C. genanigra were collected from a melon plantation in the Northwestern region of Rio Grande do Norte (RN), Brazil, and identified according to Freitas (2003). Voucher specimens were deposited at the Laboratório de Entomologia Aplicada, Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, RN, Brazil. The region of occurrence of C. genanigra has a semi-arid climate, with very hot temperatures and rainy season short and very irregular. We collected the data of temperature and relative humidity (from 2000 to 2010) at the UFERSA’s Meteorological Station, located 1.5 m from the ground, whose position coordinates are 5° 12’ 36” S latitude and 37° 18’ 43” W longitude, and altitude of 40.5 m above sea level. The average temperature in the region in that period was 27.4 °C, with an average annual precipitation of 673.9 mm and relative humidity of 68.9%.

In order to establish a laboratory colony, about 50 parental pairs were maintained in two cylindrical PVC cages (21 cm height, 10 cm diameter) at 25 ± 2 °C under a photoperiod of 12 h:12 h (L:D), relative humidity (RH) of 70 ± 10%. They were allowed continuous access to water and a diet consisting of a 1:1 (v/v) mixture of brewer’s yeast and honey (Biagioni and Freitas, 2001; Carvalho and Souza, 2000). Individuals of the fourth generation offspring were employed in the experiments.

2.2. Preimaginal development and survival

Eggs laid by females of the fourth generation were collected within 24 h with the aid of a fine brush and were transferred individually to flat-bottomed cylindrical glass tubes (8 cm high, 2.5 cm diameter). We used 60 eggs per temperature tested. The tubes with the eggs were sealed with voile tissue and were randomly allocated among seven constant temperature regimes, namely, 17, 21, 25, 29, 33, 35 or 37 °C, maintained under a 12 h:12 h (L:D) photoperiod and 70 ± 10% RH, inside climatic chambers with precision of ±1 °C. Upon hatching, and throughout preimaginal development, each larva was offered ad libitum UV-sterilized S. cereaella eggs as food. These eggs were purchased from Bug Biological Agents™. All larvae were inspected daily, and their developmental stage and survival were recorded until adult emergence. The different instars were easily separated by the presence of the exuviae inside the tubes, and the pre-pupa was differentiated from the pupa stage by the presence of a black disk inside the cocoon when the insect turns to pupa (Canard and Principi, 1984).

All the statistical analyses performed in this paper were calculated using the free statistical software R (R Development Core Team, 2009). Prior to data analysis, the assumptions of homogeneity of variances were tested by application of the Bartlett test. When data failed to meet the requirements for parametric analysis, the non-parametric Kruskal–Wallis test was employed and means were subsequently separated using the Mann–Whitney-U test. In order to interpret the data, the level of significance was set at \( P = 0.05 \) for all tests. Two-way analysis of variance was used to test the influence of temperature and sex (and their interaction) on the duration of the preimaginal stages. Contingency tables (\( \chi^2 \)-test) were used to compare the percentages of individuals completing preimaginal development at the different temperatures tested. The Bonferroni adjustment was employed to control the proportion of type-I errors (Sokal and Rohlf, 1995).

2.3. Temperature threshold and thermal requirements for preimaginal development

The relationship between temperature (\( T \)) and developmental rate (\( D \); the reciprocal of developmental time \( D \) in days) was determined from the linearization of the curve obtained in laboratory. Given the equation \( K = D(T - T_0) \), where \( T_0 \) is the lower thermal threshold for development (the temperature below which no measurable development occurs), and \( K \) is the thermal requirement (the amount of heat, expressed in degree-days for \( T > T_0 \) for completion of immature development), we have \( 1/D = a - Tb/K \) \( K = 1/K^* \). Making \( D(T) = 1/D; a = Tb/K; b = b/K; K = C0 \), the linear model is \( D(T) = a + bT \) (Bean, 1961 apud Haddad et al., 1999), in which the constants \( a \) and \( b \) were estimated by least squares regression analysis.
2.5. Egg hatchability

Samples of eggs (n = 10) that had been laid by females maintained under each of the temperature regimes were randomly selected on at least four occasions per week throughout the whole oviposition period and transferred individually to plastic Eppendorf microtubes. The eggs were kept under the same temperature regime as the parental pairs and inspected daily. The numbers of newly hatched larvae were recorded together with the number of infertile eggs (those that remained green and showed no visual signs of embryonic development) and nonviable eggs (those that became gray and showed visual signs of embryonic development but without hatching). Comparisons of the percentages of viable, nonviable and infertile eggs between the various temperature regimes were carried out using χ²-tests for equality of proportions. The durations of the embryonic periods were recorded and the data analyzed using the Kruskal–Wallis non-parametric test with means subsequently separated using the Mann–Whitney-U test.

2.6. Adult longevity and egg production

For each temperature regime studied, newly emerged adult males and females were paired, transferred to individual cylindrical PVC cages (10 cm height, 10 cm diameter) and allowed continuous access to water and the dietary mix of brewer’s yeast and honey. The water was provided inside a glass tube plugged with cotton and put upside down in the top of the cage, and the diet was provided on parafilm® strips in the wall of the cage. The pairs were maintained inside the same climatic chambers they were reared from egg to adult emergence. For the temperatures of 21, 25, 29 and 33 °C, ten pairs were used. Due to high mortality in 17, 35 and 37 °C, only five pairs were used for 35 °C, and for 17 and 37 °C no pairs were obtained.

The number of eggs laid and survival were scored daily throughout the lifespan of the female under the constant temperature applied. Regression curves were plotted for the pre-oviposition (the period between emergence and the beginning of egg production), total oviposition (the period from the first oviposition to the last), effective oviposition (only the days in which the female oviposited) and post-oviposition (the period between the end of oviposition and the death of the female) periods. Weibull distributions for survival probability to compare the longevity of males and females and the death of the female) periods. Weibull distributions for survival probability to compare the longevity of males and females at the studied temperatures were also calculated and the not significantly different survival curves were grouped using contrasts.

3. Results and discussion

3.1. Effect of temperature on preimaginal survival and developmental time

None of the C. genanigra larvae survived to complete their development at the highest temperature tested, i.e. 37 °C, and hence no results are available for this temperature. Temperatures within the range 17–35 °C exerted a significant effect (146.906 ≤ F ≤ 4555.440; df = 4, 164; P < 0.001) on preimaginal developmental times (Table 1), which decreased from 17 to 33 °C but increased between 33 and 35 °C in almost all stages. This increase, taken together with the observed survival of only 18.3% at 35 °C (Table 2), leads to the conclusion that this temperature is too high for the species and is, apparently, detrimental to its metabolism. Kuznetsova (1969) has previously reported increases in mortality of the preimaginal stages of Chrysoperla carnea Stephens at 35 °C, what shows this temperature to be limiting for lacewings development.

The sex also affected significantly the preimaginal developmental time for 1st and 2nd instars, prepupa, pupa and total (3.982 ≤ F ≤ 32.776; df = 1, 164; P < 0.05), but did not affect the egg and 3rd instar developmental times (0.995 ≤ F ≤ 1.307; df = 1, 164; P > 0.05) (Table 1). No significant interaction between temperature and sex was observed (0.401 ≤ F ≤ 2.027; df = 4, 164; P > 0.05). The period required for development from egg to adult ranged from approximately 63 days for females maintained at 17 °C to 14 days for males at 33 °C (Table 1). Total preimaginal developmental times for males were significantly shorter than those of females at 21 and 29 °C, but were similar for the two sexes at 25, 33 and 35 °C. At 17 °C, no males developed and hence no comparative data are available for this temperature. Significant differences between males and females with respect to larva development times

Table 2
The effect of temperature on the number of individuals of C. genanigra that completed preimaginal development when maintained under a 12 h:12 h (L:D) photoperiod, 70 ± 10% relative humidity, and fed on S. cerealella eggs.

<table>
<thead>
<tr>
<th>Incubation temperature (°C)</th>
<th>Number of individuals completing development</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td>21</td>
<td>43</td>
<td>71.7</td>
</tr>
<tr>
<td>25</td>
<td>46</td>
<td>76.7</td>
</tr>
<tr>
<td>29</td>
<td>39</td>
<td>65.0</td>
</tr>
<tr>
<td>33</td>
<td>35</td>
<td>58.3</td>
</tr>
<tr>
<td>35</td>
<td>11</td>
<td>18.3</td>
</tr>
</tbody>
</table>

For each sex, means within the column bearing different letters are significantly different according to Mann–Whitney-U test (P < 0.05).

Table 1
The effect of temperature on the development times of the preimaginal stages of C. genanigra maintained under a 12 h:12 h (L:D) photoperiod, 70 ± 10% relative humidity, and fed on S. cerealella eggs. N = Number of individuals observed. ND = values not determined.

<table>
<thead>
<tr>
<th>Incubation temperature (°C)</th>
<th>N</th>
<th>Developmental time (days) ± standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Egg</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>4</td>
<td>8.8 ± 0.25a</td>
</tr>
<tr>
<td>21</td>
<td>28</td>
<td>5.8 ± 0.08b</td>
</tr>
<tr>
<td>25</td>
<td>21</td>
<td>3.3 ± 0.11c</td>
</tr>
<tr>
<td>29</td>
<td>17</td>
<td>3.0 ± 0.00d</td>
</tr>
<tr>
<td>33</td>
<td>21</td>
<td>2.0 ± 0.00f</td>
</tr>
<tr>
<td>35</td>
<td>5</td>
<td>2.6 ± 0.24e</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>21</td>
<td>15</td>
<td>5.7 ± 0.13a</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>3.2 ± 0.09b</td>
</tr>
<tr>
<td>29</td>
<td>22</td>
<td>3.0 ± 0.00c</td>
</tr>
<tr>
<td>33</td>
<td>14</td>
<td>2.0 ± 0.00e</td>
</tr>
<tr>
<td>35</td>
<td>6</td>
<td>2.3 ± 0.21d</td>
</tr>
</tbody>
</table>
were observed at 33 °C for 1st instar larvae and at 29 and 35 °C for 2nd instar larvae. For the prepupal stage, three (21, 25 and 29 °C) of the five temperatures showed differences between males and females, and for the pupal stage, two temperatures (21 and 25 °C). We can observe that the major differences occur in the cocoon stage (prepupa + pupa). These results corroborate the hypothesis of Canard and Principi (1984) that the pupal period depends on the sex of the individual as well as on abiotic factors, but they also mention that this temperature dependent protandry is rather obscure since it is not a general trend in lacewings, as we can observe in the present study for *C. genanigra*. Pappas et al. (2008) evaluating the preimaginal development of males and females of *Dichochrysa prisina* Burmeister, also observed significant differences in preimaginal development between sexes in three of five tested temperatures, what leads to conclude that this is not a rule, and females do not always develop lower than males.

Temperature also elicited a significant effect ($\chi^2 = 99.46; df = 5, P < 0.05$) on the percentage of individuals that successfully completed preimaginal development (Table 2). At the lowest (17 °C) and highest (35 °C) temperatures employed, the survival percentages were, respectively, 6.7% and 18.3%, values that were significantly lower than those attained at the other temperatures studied ($P < 0.05$). In the temperature range from 21 to 33 °C, the percentages of individuals that completed their preimaginal development varied from 58.3% to 76.7%, without significance among them ($\chi^2 = 6.60; df = 3, P > 0.05$).

Previous studies involving *Chrysoperla externa* (Hagen) (Figueira et al., 2000) and *Chrysoperla raimundoi* (Freitas & Penny) (Pessoa et al., 2009) have revealed that the pupal stage is the most susceptible to low temperatures. These findings were confirmed in the present study in which the survival rate for pupae was 20% at 17 °C but rose to 92% at 25 °C.

### 3.2. Temperature threshold and thermal requirements for preimaginal development

The preimaginal developmental rates for *C. genanigra* were related linearly to temperature over the range 17–35 °C ($P < 0.05$)
(Table 3). The theoretical temperature threshold ($T_b$) for total preimaginal development was approximately 10.8 °C, and this is very close to the thresholds of 10.9 and 10.7 °C, respectively, found in C. externa (Maia et al., 2000; Figueira et al., 2000), and of 11.3 °C for C. raimundoi (Pessoa et al., 2009). The thermal requirement ($K$) of C. genanigra for total development was 336.7 degree-days above 10.8 °C, a value that is very similar to 335.3 degree-days (Pessoa et al., 2009). These information show that even C. genanigra being from a semi-arid climate, it has almost the same thermal requirements of other Chrysoperla species from cooler regions of Brazil.

3.3. Effect of temperature on adult longevity and egg production

The increase in temperature reduced the duration of oviposition, effective oviposition and post-oviposition periods of C. genanigra (Fig. 1). The pre-oviposition period also decreased steadily from 75.2 and 66.0 days at 21°C to 0.9 and 1.0 day at 35°C, respectively. These reductions were expected, once the increase in temperature accelerates the physiology of insects. The increase in duration of pre-oviposition when temperature arises from 33°C to 35°C may probably be caused by negative effects of such high temperature in the reproductive tracts of the females.

The durations of oviposition and effective oviposition observed at 25 °C, i.e. 47.1 and 42.3 days, respectively, were shorter than for C. externa fed with eggs of Anagasta kuehniella (Zeller) (71.4 and 62.7 days) (Ribeiro and Carvalho, 1991). On the other hand, the pre-oviposition period decreased progressively from 75.2 and 66.0 days, respectively, at 21 °C to 13.2 and 11.2, respectively, at 35 °C. The post-oviposition period decreased from 3.6 days at 21°C to 0.9 and 1.0 day at 33°C and 35°C, respectively. These reductions were expected, once the increase in temperature accelerates the physiology of insects. The increase in duration of pre-oviposition when temperature arises from 33°C to 35°C may probably be caused by negative effects of such high temperature in the reproductive tracts of the females.

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The temperature also had significant effect on the number of eggs produced per female of C. genanigra (Table 4). The highest egg production was attained at 25 °C, with an average of 992.7 eggs laid per female and a mean egg viability of 75.3%. The lowest numbers of eggs (151.4 and 30.5 per female, respectively) were recorded at the highest tested temperatures, namely, 33 and 35 °C (Table 4). Eggs laid at 35 °C, however, had no stalks and were laid directly onto the substrate, probably due to excess of heat for silk solidification. The average numbers of eggs laid per female at 21 and 29 °C were not significantly different from each other, and were substantially less than half of that produced at the optimum temperature.

Experiments in which C. raimundoi larvae were fed with S. cerealella eggs and adults with a 1:1 brewer’s yeast and honey mix diet, revealed a much lower egg production at 25 °C for this lacewing, with an average of 451.4 eggs per female and a mean viability of 85.9% (Pessoa et al., 2009). The egg production observed here for C. genanigra is much higher than the production of C. raimundoi. The number of eggs produced (336.4 per female) by C. carnea maintained at 28 °C and fed with Rhopalosiphium maidis (Fitch) (El-Serafi et al., 2000), was very similar to that observed in the present study for C. genanigra at 29 °C. These results show that C. genanigra is highly competitive in egg production when compared with other studied species, which means that the use of exotic predators for whitefly IPM programs in melon crops is not necessary.

Survival analysis (Fig. 2) revealed that for each temperature tested, male and female survival were not significantly different ($P > 0.05$) but their rate of development varied significantly ($P < 0.05$) according to temperature. Owing to the small number of pairs obtained at 35 °C, it was not possible to plot a curve of survival probability for this temperature, but the average longevity of males and females were 21 and 24 days, respectively, for that temperature. Weibull survival analysis showed that the time for half the population of insects is dead was approximately 109, 53, 33 and 24 days for the temperatures of 21, 25, 29 and 33 °C, respectively. These results, together with the oviposition period, can help in calculating the time to renew the populations in laboratory rearing.

3.4. Effect of temperature on egg viability

The viabilities (hatchabilities) of eggs laid by females of C. genanigra were affected significantly by the temperature at which preimaginal development had been completed ($\chi^2 = 135.76; df = 4, P < 0.05$). The lowest viabilities of 0% and ~44% were recorded at 35 and 21 °C, respectively, while viabilities ranged from approximately 65% to 75% at the other tested temperatures (Table 4). Temperature also affected significantly the percentages of nonviable

Table 4

<table>
<thead>
<tr>
<th>Incubation temperature (°C)</th>
<th>$Ne$</th>
<th>Number of eggs per female ± standard error</th>
<th>$Ne$</th>
<th>Viable eggs (%)</th>
<th>Nonviable eggs (%)</th>
<th>Infertile eggs (%)</th>
<th>Duration of embryonic development (days) ± standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>10</td>
<td>418.1 ± 45.97B</td>
<td>442</td>
<td>43.7</td>
<td>3.8</td>
<td>52.5</td>
<td>6.7 ± 0.09A</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>992.7 ± 75.11A</td>
<td>434</td>
<td>75.3</td>
<td>8.1</td>
<td>16.6</td>
<td>3.8 ± 0.07B</td>
</tr>
<tr>
<td>29</td>
<td>10</td>
<td>344.2 ± 71.52B</td>
<td>196</td>
<td>65.3</td>
<td>4.6</td>
<td>30.1</td>
<td>2.7 ± 0.15C</td>
</tr>
<tr>
<td>33</td>
<td>10</td>
<td>151.4 ± 31.01C</td>
<td>151</td>
<td>64.9</td>
<td>12.6</td>
<td>22.5</td>
<td>2.4 ± 0.17C</td>
</tr>
<tr>
<td>35</td>
<td>5</td>
<td>30.5 ± 4.17D</td>
<td>114</td>
<td>0.0</td>
<td>15.8</td>
<td>84.2</td>
<td>–</td>
</tr>
</tbody>
</table>

Mean values in the column bearing different uppercase letters are significantly different according to Mann–Whitney-U test ($P < 0.05$).

Fig. 2. Weibull distributions for survival probability of C. genanigra males and females accumulated at each of the four temperatures.
(χ² = 62.40; df = 4, P < 0.05) and infertile (χ² = 347.90; df = 4, P < 0.05) eggs produced. The highest numbers of nonviable eggs were recorded at 33 and 35 °C (12.6% and 15.8%, respectively), while the lowest number of unfertile eggs was recorded at 25 °C (16.6%). The duration of the embryonic period of eggs varied according to temperature, and decreased gradually from 6.7 days at 21 °C to 2.4 days at 33 °C.

The results presented herein demonstrate that C. genanigra can be readily mass reared on S. cerealella eggs, and that adults can be successfully maintained on a dietary mix of brewer's yeast and honey, using technologies similar to those that have been previously employed for other Chrysopeidae species. Additionally, C. genanigra can develop and reproduce at temperatures within the range 21–33 °C, although the best temperature for egg production in the laboratory was determined to be 25 °C. We can also conclude that despite the fact that the optimum temperature for C. genanigra is 25 °C, the environmental temperature is higher than that. This shows that C. genanigra is better adapted than other species, maybe in an ecological way, to the environmental conditions, once it occurs in field even in the hottest and dryer months, while other do not (Bezerra et al., 2010).

4. Role of funding agency

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