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Source: Florida Entomologist, 101(3) : 458-463

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.101.0303>

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# Effects of temperature on the development of *Podisus nigrispinus* (Heteroptera: Pentatomidae): implications for mass rearing

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## Abstract

Predatory stinkbugs such as *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) have been mass-reared in the laboratory and released for use in integrated pest management programs. However, the rearing of this natural enemy may not coincide with pest outbreaks in the field, which indicates the need for techniques to manipulate the life cycle of the predator. The objective of this study was to evaluate the effect of different temperatures on the development and other biological characteristics of the stinkbug predator *P. nigrispinus*. Six temperatures were used (13, 17, 21, 25, 29, and 33 °C ± 0.2 °C), and the following parameters were evaluated: duration of egg and nymphal stages; survival rate of the nymphal stages and newly emerged adults; adult longevity; number of eggs per female; pre-oviposition, oviposition, and post-oviposition periods; and adult weight. At 4 temperatures it was possible to obtain survival at all nymphal stages. Longer adult longevity, pre-oviposition, oviposition, and post-oviposition periods were obtained at 17 °C. However, the number of eggs, weight, and size were negatively affected by this cooler temperature. Temperatures of 21 and 25 °C were the most appropriate for the development of the predator; however, the temperatures 17 and 29 °C allowed delay or acceleration of the predator's life cycle, thereby facilitating release of the predators when they are needed in the field for biological suppression of pests.

Key Words: insect; Asophinae; biological control; stinkbug predator

## Resumen

Percevejos predadores, como o *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae), têm sido criado massalmente em laboratório e liberado nos programas de Manejo Integrado de Pragas. No entanto, a produção de inimigos naturais pode não coincidir com o surto de pragas no campo, fazendo necessário o uso de técnicas que permitam manipular o ciclo de vida do predador. Objetivou-se, portanto, investigar os efeitos de diferentes temperaturas no desenvolvimento e em outras características biológicas do percevejo predador *P. nigrispinus*. Seis temperaturas foram utilizadas (13, 17, 21, 25, 29, e 33 °C ± 0,2), e os parâmetros avaliados foram: duração da fase de ovo e dos estádios ninfais; sobrevivências; longevidade de adultos; número de ovos por fêmea; período de pré-oviposição, oviposição e pós-oviposição; peso e tamanho de adultos. Em quatro temperaturas foi possível obter a duração e sobrevivência em todos os estádios ninfais. Maior longevidade de adultos e período de pré-oviposição, oviposição e pós-oviposição foram obtidos na temperatura de 17 °C. Todavia, o número de ovos, peso e tamanho de adultos foram afetados adversamente. As temperaturas de 21 e 25 °C são as mais apropriadas para o desenvolvimento do predador. Por outro lado, as temperaturas de 17 e 29 °C permitem retardar ou acelerar o ciclo de vida do predador mediante a necessidade de inimigos naturais no campo.

Palavras Chave: inseto; Asophinae; controle biológico; percevejo predador

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*Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) is a generalist predator found in temperate and tropical regions (Neves et al. 2010; Inayat et al. 2011). It is an efficient predator on several pests, such as *Alabama argillacea* Hübner (Lepidoptera: Noctuidae), *Anticarsia gemmatilis* Hübner (Lepidoptera: Noctuidae), and *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), in crops such as cotton (Malvaceae), tomato (Solanaceae), soybean (Fabaceae), and eucalyptus (Myrtaceae) (Matos-Neto et al. 2002; Oliveira et al. 2002; Torres et al. 2002; Zanuncio et al. 2002). This predator has been mass-reared in the laboratory and then released for biological control in integrated pest management programs (Zanuncio et al. 2002; Bottega et al. 2014).

Although laboratory mass-rearing produces a large number of natural enemies, the production of predators may not coincide with pest outbreaks in the field (Costa et al. 2016), which generates costs once the prey is created to continuously sustain production throughout the yr (Neves et al. 2010). The lack of timing in the production schedule of natural enemies with the appearance of pests in the field indicates the need for storage techniques (Colinet & Hance 2010) or methods that manipulate the life cycle of the biological control agent.

It is known that the development and survival of an insect may vary in response to biotic and abiotic factors (Courret et al. 2014). In addition, temperature is the principal factor that explains the develop-

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mental rate of insects (Liu et al. 1995; Kingsolver & Huey 2008) due to the changes in metabolic rates (Hodkova & Hodek 2004). For example, higher temperatures are associated with an increased rate of development (Kingsolver & Huey 2008; Arrese & Soulages 2010) that is ideal when there is a fast need for natural enemies, whereas the use of low temperatures has been proven as a method that delays development (Colinet & Hance 2010). However, the temperature manipulation for either the delay in the life cycle or obtaining the predators quickly is not so simple. Prolonged exposure to cold temperatures may cause damage that, once progressively accumulated, may become irreversible or lethal (Košťál et al. 2006; Silva et al. 2013), as in *P. nigrispinus*, in which the eggs are lethally affected at 5 °C (Vacari et al. 2014).

In general, embryos are rather tolerant of low temperatures (Baek et al. 2014), and under such conditions nymphs may hatch; however, the development cycle may not be completed, as in *Edessa meditabunda* (Fab.) (Hemiptera: Pentatomidae) subjected to 15 °C, in which the nymphs survived only until the second instar (Gonçalves et al. 2008). Negative effects also are experienced when nymphs are subjected to temperatures near the upper limit (Haddad et al. 1999). In *Podisus distinctus* (Stål) (Hemiptera: Pentatomidae), the nymphal duration decreased with the increase of the temperature, but 33 °C was lethal to the insects (Santos et al. 2004).

Because the mortality and developmental rate are both strongly affected by low and high temperatures, it is very important to consider temperatures in studies of mass rearing. Thus, the objective of this study was to evaluate the effects of different temperature ranges on the development and other biological characteristics of *P. nigrispinus* in order to optimize mass rearing.

## Materials and Methods

### INSECTS

The tests were carried out at the Forest Entomology Laboratory in the Forestry Sciences Department, located in the Federal University of Sergipe, Sergipe, Brazil. The insects were obtained from the same laboratory, where they were kept under the conditions of 25 ± 0.5 °C, 60 ± 9.5% RH, and 12:12 h (L:D) photoperiod. The adult *P. nigrispinus* were reared in screened cages of 60 cm × 40 cm × 40 cm, and fed with the pupae of its alternative prey *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) (previously obtained from mass rearing in the same laboratory). Water was provided through moistened cotton, which was deposited on the top screen of the cage.

The eggs deposited by the females in the cages were collected daily with the aid of cotton ball on the oviposition dish, and then about 30 eggs were transferred to each of the Petri dishes (9.0 cm diam × 1.5 cm H) containing a moistened cotton plug with distilled water. After hatching, the nymphs remained in these cages and were fed with *T. molitor* pupae until the adult stage, and then with the aid of forceps all the adult insects obtained were transferred to the cages (60 cm × 40 cm × 40 cm) for mating.

### DEVELOPMENT OF *PODISUS NIGRISPINUS* AT DIFFERENT TEMPERATURES

From the colony, 400 females were separated in acrylic boxes of 11 cm × 11 cm × 3.5 cm (10 females per box) 24 h before collecting the eggs. The egg masses of up to 24 h old were collected from the Petri dishes and then separated later by treatment. The treatments consisted of 6 constant temperatures (13, 17, 21, 25, 29, and 33 °C with SE ± 0.2 °C), 60 ± 9.5% RH, and 12:12 h (L:D) photoperiod. Each treat-

ment was initiated with 25 replicate Petri dishes, each with 20 eggs. After hatching, the nymphs were kept in the same Petri dishes and fed with *T. molitor* pupae.

After completing the nymphal stage, 20 pairs (1 male and 1 female) of newly emerged adults were separated in acrylic boxes and maintained in the same conditions as described previously (temperatures, relative humidity, and photoperiod). A wet cotton ball was deposited on the top of the acrylic box in order to maintain humidity, and the insects were fed with *T. molitor* pupae.

Daily observations were made in order to maintain an adequate water supply, as well as to make observations (by the usage of a stereoscopic microscope) of the evaluated parameters that consisted of (a) duration of egg and nymphal stages (determined by changes in morphology of the insects); (b) survival rate on the nymphal stages and newly emerged adults; (c) male and female longevity; (d) number of eggs (per female); (e) pre-oviposition, oviposition, and post-oviposition periods; and (f) weight of newly emerged males and females.

### STATISTICAL ANALYSES

All parameters were evaluated with analysis of variance (1-way ANOVA) and then to regression analysis, with the non-linear model selected according to the significance of the regression coefficients ( $t$ ,  $P < 0.05$ ) and the coefficient of determination ( $R^2$ ). The statistical analyses were conducted using SISVAR 5.0 software (Ferreira 2014).

## Results

The duration of the egg stage was longest and shortest when maintained at 13 and 33 °C, respectively. However, these temperatures did not allow the development of the nymph to the second instar. Thus, based only on the temperatures that allowed complete development, the longest and shortest duration in *P. nigrispinus* developmental periods occurred at 17 and 29 °C. The mean (± SE) total development times (egg to adult) were 65.3 ± 2.0; 38.4 ± 0.5; 21.5 ± 0.4; and 17.4 ± 0.3 when reared at 17, 21, 25, and 29 °C, respectively (Fig. 1).

Higher levels of *P. nigrispinus* survival were obtained between 21 and 25 °C. The survival rates of the first instar were reduced at extreme temperatures (13 and 33 °C). In the other instars there was no survival at 13 and 33 °C; however, at 17 and 29 °C the survival rate decreased as well. This trend was evident when the quadratic regression equations were evaluated (Fig. 2).

In the nymph and adult stages, the maximum survival rates occurred between 21 and 25 °C. However, the survival rate diminished over time, with the proportion surviving highest during the first instar (75 to 90%), and lowest during the adult stage (25 to 50%) (Fig. 2A and 2F).

The longevity of *P. nigrispinus*, both male and female, decreased as the temperature increased independent of the temperature, females had a shorter longevity than males (Fig. 3A). With respect to egg production, insects reared at 17 °C produced a low number of eggs per female (13.5 ± 6.6), whereas maximum egg production per female (208.1) occurred at 24.38 °C (Fig. 3B).

The pre-oviposition period of *P. nigrispinus* was 27.6 ± 0.9, 8.4 ± 0.9, 8.0 ± 0.5, and 7.5 ± 0.3 d for insects reared at 17, 21, 25, and 29 °C, respectively. The oviposition and post oviposition periods also decreased with increasing temperature (Fig. 4).

Rearing temperatures also affected the weight of both males and females. The weight of *P. nigrispinus* males was higher with increasing temperature; however, the weight of females at extreme temperatures (17 and 29 °C) decreased. It also was observed that females were on average 17.5 mg heavier than males (Fig. 5).

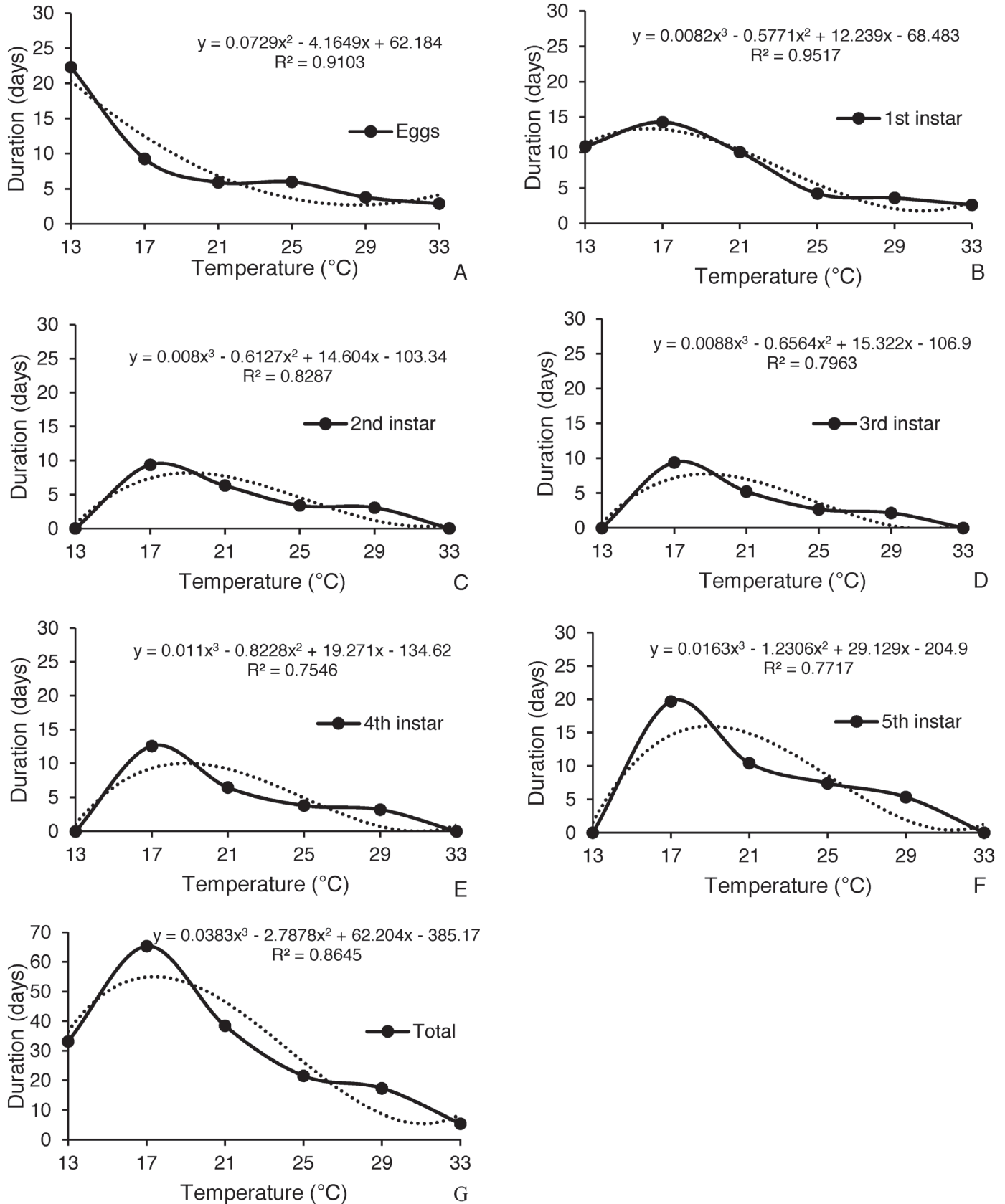
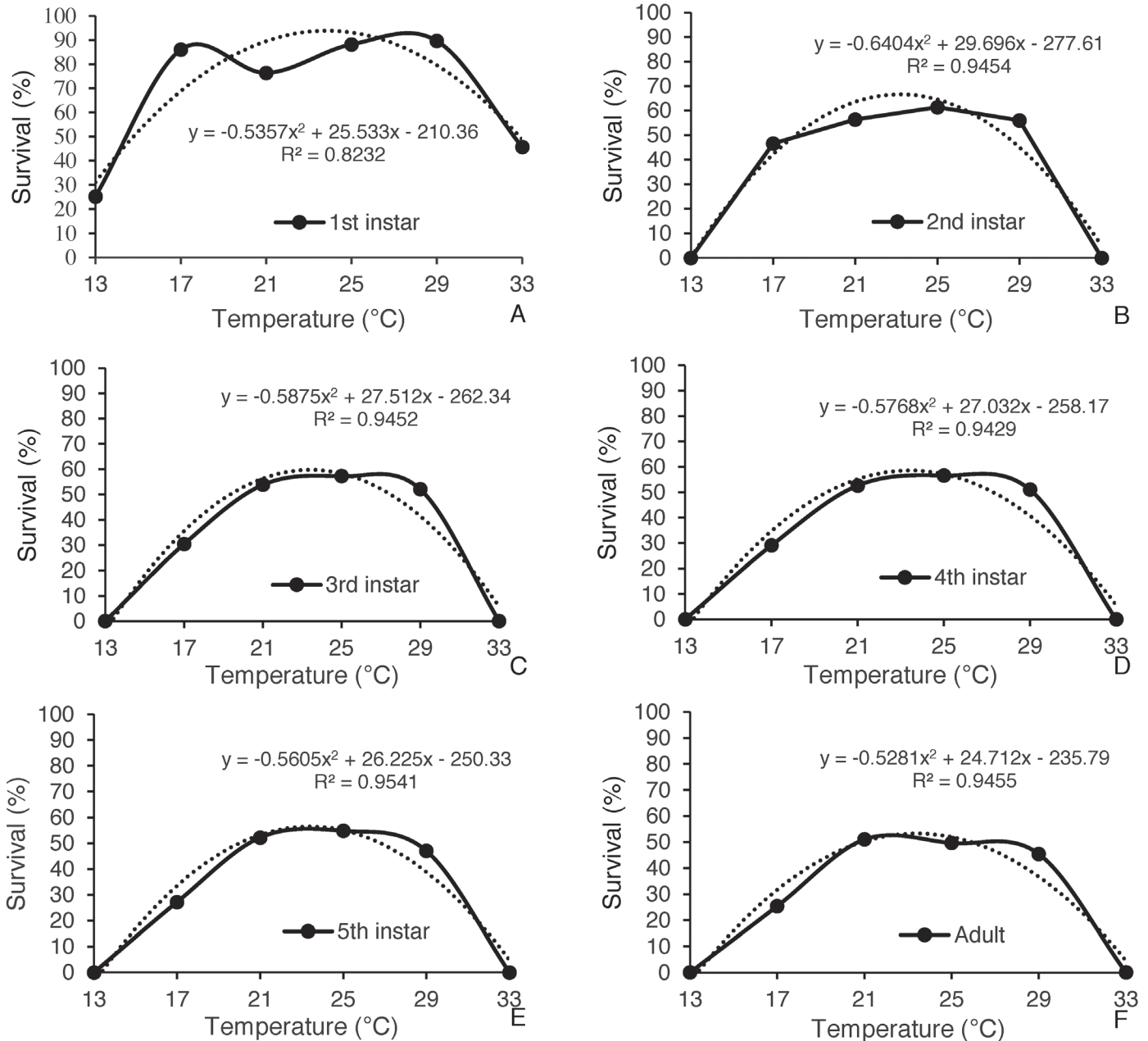


Fig. 1. Duration (d) of the egg stage (A), nymphal stages (B, C, D, E, and F), and total development of nymphal stage (G) of *Podisus nigrispinus* (Heteroptera: Pentatomidae) subjected to constant temperatures (13, 17, 21, 25, 29, and 33 ± 0.2 °C).



**Fig. 2.** Survival (%) in nymphal stages (A, B, C, D, and E) and newly emergent adults (F) of *Podisus nigrispinus* (Heteroptera: Pentatomidae) subjected to constant temperatures (13, 17, 21, 25, 29, and 33 ± 0.2 °C).

## Discussion

The development period of insects tends to increase with the decreasing temperature up to a certain limit, due to the reduction of metabolic rates (Hodkova & Hodek 2004), and this also was observed in all stages of the life cycle of *P. nigrispinus*. Although there were some negative aspects to rearing the insects at lower temperatures, this procedure of rearing at lower temperatures might allow increased production of predators in the absence of pest insects in the field.

Temperatures near the thermal limits of the insect must be avoided (Baek et al. 2014), because this can affect them lethally. For example, although there was some growth at 13 and 33 °C, the insects eventually died in the nymphal stage. The lower limit for *P. nigrispinus* was previously defined as 11.5 °C (Didonet et al. 1995), but in this study 13 °C was lethal.

The most suitable temperature for delaying nymphal development was 17 °C because the developmental cycle was 3.0 and 3.7 times longer than at 25 and 29 °C, respectively (Fig. 1). In a similar study of the development of *P. distinctus* when cultured at temperatures varying from 17 and 33 °C, extension of the nymphal period also was demonstrated, and nymphal development time at 17 °C was 4.5 times longer than at 29 °C (Santos et al. 2004).

Increase in development time caused by the reduction in metabolic rates can cause the energy resources of the insect to be allocated to its survival (Vacari et al. 2014), and this was clearly observed by the decrease of parameters such as nymphal survival (Fig. 2), oviposition (Fig. 4), and weight of newly emerged male and female (Fig. 5). The same trade-off was observed in the storage of *P. nigrispinus* eggs, wherein insects from eggs stored for 17 d at 15 °C displayed a decreased the nymphal period, nymphal viability, and weight (Vacari et al. 2014).



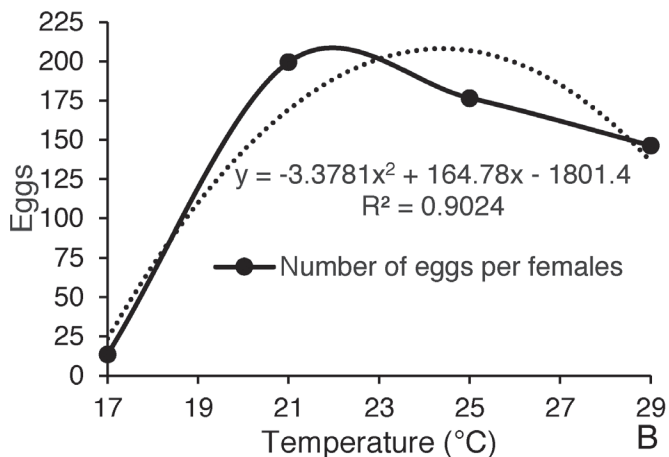
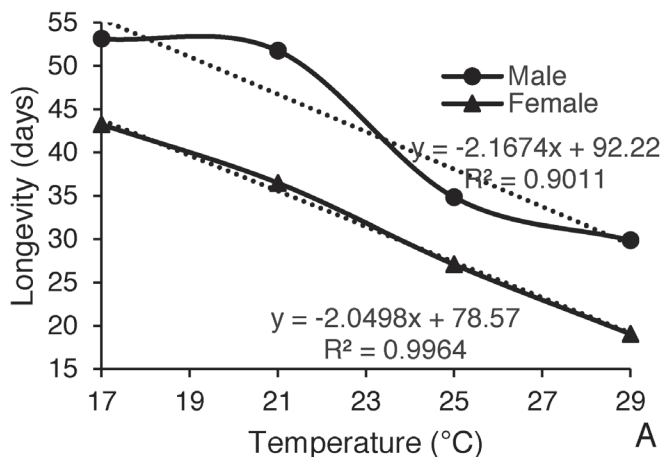


Fig. 3. Male and female longevity (d) (A) and number of eggs per female (B) of *Podisus nigrispinus* (Heteroptera: Pentatomidae) subjected to constant temperatures (13, 17, 21, 25, 29, and 33 ± 0.2 °C). \*In the 13 and 33 °C temperatures there was no adult emergence.

Temperature affects the development (Baek et al. 2014; Poncio et al. 2016; Johnson et al. 2016) and reproduction of insects (Scriber & Slansky Jr 1981), as can be observed when the insects were cultured at 17 °C, which caused an increase in the longevity of males and females (Fig. 3A). However, this resulted in a reduction in the total number of eggs per females (13.5 ± 6.6 eggs per female) (Fig. 3B) and in mating. Low temperatures can reduce energy reserves of insects due to the low food consumption (Renault et al. 2003), which indicates that both high longevity and low reproduction obtained in this study are associated not only with the allocation of energy expenditure for survival, but also with limited sources of energy.

Higher temperatures also influenced the reproduction of *P. nigrispinus*, resulting in a decrease in the pre-oviposition, oviposition, and post-oviposition periods (Fig. 4). Results similar to these were found when *P. nigrispinus* was fed with *Alabama argillacea* (Hübner) Lepidoptera: Noctuidae) under different temperatures (Medeiros et al. 2003). According to the literature, fecundity of *P. nigrispinus* is high at 25 °C (Medeiros et al. 2003). However, even at ideal temperatures, total egg production per female was low (199.4 ± 35.7 and 176.5 ± 33.0, at 21 and 25 °C, respectively). This may be due to the fact that females that have access to only 1 male may experience a scarcity of spermatozoa (Torres & Zanuncio 2001). However, those that perform

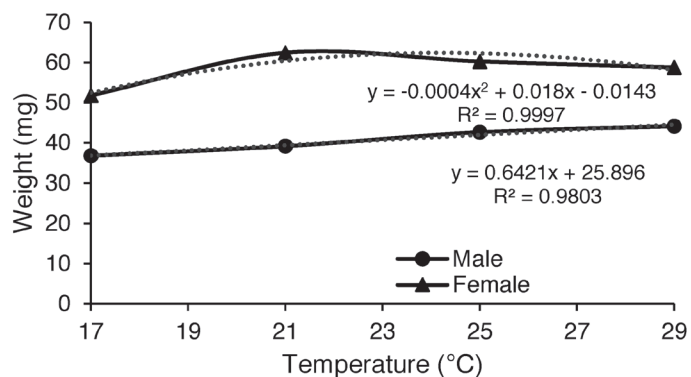


Fig. 5. Weight (mg) of newly emerged *Podisus nigrispinus* (Heteroptera: Pentatomidae) males and females subjected to constant temperatures (13, 17, 21, 25, 29, and 33 ± 0.2 °C). \*In the 13 and 33 °C temperatures there was no adult emergence.

the multiple coupling (with several males) may produce a higher number of eggs, which can vary from 350 to 390 (Oliveira et al. 2002, 2011; Espindula et al. 2010).

Until the fourth instar, male and female nymphal weights are not significantly different; however, in the fifth instar females become heavier than males. The greater weight of females as compared to males is due to the accumulation of fat (which is necessary for reproduction) and also due to the development of the ovaries and eggs (Oliveira et al. 2002, 2011).

This study demonstrated that the best developmental conditions for this predator were 21 and 25 °C. At 13 and 33 °C, the nymphs died in the first instar. However, the optimal temperature for mass rearing was 29 °C. Should it be desirable to delay the development of the predators so as to increase availability of predators at a certain time, they can be cultured at 17 °C, although there are trade-off with this temperature, as reflected in reduced survival, number of eggs per female, weight, and size of the predator.

### Acknowledgments

The authors would like to thank the “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)” for financial support.

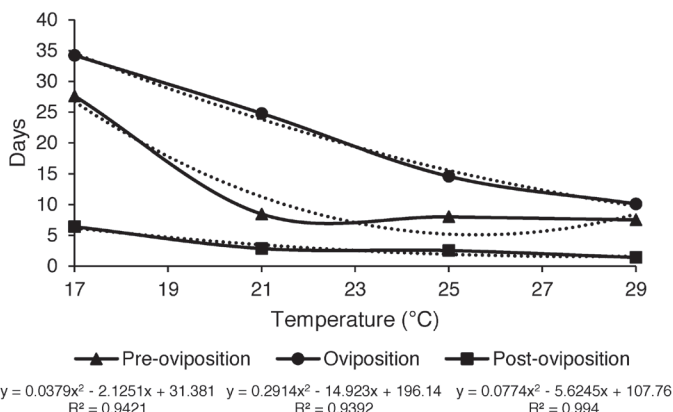


Fig. 4. Pre-oviposition, oviposition, and post-oviposition periods of *Podisus nigrispinus* (Heteroptera: Pentatomidae) subjected to constant temperatures (13, 17, 21, 25, 29, and 33 ± 0.2 °C). \*In the 13 and 33 °C temperatures there was no adult emergence.

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