



## **Mating Preferences and Consequences of Choosing Sibling Or Non-Sibling Mates by Females of the Predator *Podisusnigrispinus* (Heteroptera: Pentatomidae)**

Authors: Poderoso, Júlio César Melo, Zanuncio, José Cola, Puker, Anderson, Ramalho, Francisco De Souza, Wilcken, Carlos Frederico, et al.

Source: Florida Entomologist, 96(2) : 419-423

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.096.0259>

---

BioOne Complete ([complete.bioone.org](https://complete.bioone.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

## MATING PREFERENCES AND CONSEQUENCES OF CHOOSING SIBLING OR NON-SIBLING MATES BY FEMALES OF THE PREDATOR *PODISUS NIGRISPINUS* (HEMiptera: PENTATOMIDAE)

JÚLIO CÉSAR MELO PODEROSO<sup>1</sup>, JOSÉ COLA ZANUNCIO<sup>1,\*</sup> ANDERSON PUKER<sup>1</sup>, FRANCISCO DE SOUZA RAMALHO<sup>2</sup>,  
CARLOS FREDERICO WILCKEN<sup>3</sup> AND ERALDO LIMA<sup>1</sup>

<sup>1</sup>Departamento de Entomologia, Universidade Federal de Viçosa, 36570-000 Viçosa-Minas Gerais, Brazil

<sup>2</sup>Unidade de Controle Biológico, EMBRAPA Algodão, 174, 58107-720, Campina Grande, Paraíba, Brazil

<sup>3</sup>Departamento de Produção Vegetal, Faculdade de Ciências Agrônômicas, Universidade Estadual Paulista, 18603-970, Botucatu, São Paulo, Brazil

\*Corresponding author: E-mail: zanuncio@ufv.br

### ABSTRACT

The sex with the greater investment in offspring is more careful in selecting sexual partners. Mating preferences and consequences of choosing sibling or non-sibling males by *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) females were investigated. Females of this predator were placed with sibling, non-sibling or both types of males in 500 mL plastic containers. The numbers of matings and egg masses per female were registered after 12 h. Eggs were transferred to Petri dishes (9.5 × 1.5 cm) and the number of nymphs that hatched were recorded daily until 10 days after each oviposition. Female longevity with sibling, non-sibling or both types of males was registered and adults were weighed and measured after their deaths. Females of *P. nigrispinus* did not discriminate between sibling or non-sibling males, which can lead to inbreeding. However, the greater number of eggs laid and the higher egg viability of *P. nigrispinus* females that mated with non-sibling males indicate that this predator can avoid inbreeding depression including reduced fitness by mating with unrelated males.

Key Words: biological control, choice mate, sexual selection, stinkbug

### RESUMO

O sexo que mais investe na prole é mais seletivo na escolha dos parceiros sexuais. Preferências e consequências da escolha de parentes (irmãos) ou não-irmãos por fêmeas de *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) foram estudadas. Fêmeas desse predador foram colocadas com machos irmãos ou não-irmãos em recipientes plásticos (500 ml). O número de acasalamentos e o de massas de ovos foram registrados por fêmea em intervalos de 12 horas. Os ovos foram transferidos para placas de Petri (9,5 × 1,5 cm) e o número de ninfas eclodidas observado, diariamente, até 10 dias após cada oviposição. A longevidade de fêmeas copuladas com machos irmãos, não-irmãos ou ambos foi registrada e após a morte, os adultos foram pesados e medidos. Fêmeas de *P. nigrispinus* não discriminam entre machos irmãos ou não-irmãos, indicando que este predador pode evitar a endogamia incluindo redução da aptidão por meio de cruzamentos com machos não relacionados.

Palavras Chave: controle biológico, escolha de cópula, percevejo, seleção sexual

Pentatomid predators (Hemiptera: Pentatomidae) have been evaluated for the augmentative biological control of insect pests (Tipping et al. 1999), which necessitates the study of their biology to develop mass rearing methodologies (Zanuncio et al. 2001). The predator *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae) is common in the Americas and in several agroecosystems in Brazil where it is an important biological control agent of defoliating caterpillars

and other insects (Molina-Rugama et al. 1997; Medeiros et al. 2003; Torres & Zanuncio 2001).

*Podisus nigrispinus* is polyandrous and its females perform up to 13 copulations with 3 to 4 males over a 1-2 wk period, which is sufficient to fertilize its eggs and to produce nymphs for over 80% of its lifetime (Torres & Zanuncio 2001). Costs and benefits of reproduction are asymmetric for males and females, resulting in sexual conflict (Watson et al. 1998). Mate

choice can affect reproductive success and females may have multiple copulations to maximize their offspring (Ridley 1990). This behavior may increase female gains in genetic and nutritional material (Reynolds 1996) resulting in higher fertility and offspring heterogeneity (Koshiyama et al. 1996). Disadvantages to multiple mating include: 1) energy expenditure, 2) increased risk of predation (Crudington & Siva-Jothy 2000); 3) contamination by pathogens (Rolff & Siva-Jothy 2002) and 4) toxic effects of substances transferred during ejaculation (Chapman et al. 1995).

Females of *P. nigrispinus* with several long copulations are more fecund (Torres & Zanuncio 2001; Sousa-Souto et al. 2006; Rodrigues et al. 2009), but consequences of multiple mating remain unclear. The objective of this research was to elucidate female preference for mates and to determine the consequences of these preferences.

#### MATERIALS AND METHODS

The experiment was conducted under laboratory conditions at the Laboratory of Biological Control of the Department of Animal Biology of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil in a climate chamber at  $28 \pm 1$  °C,  $70 \pm 10\%$  R.H. and 12:12 h L:D.

##### Insect Culture

Eggs of *P. nigrispinus* were obtained from different cages from rearing facilities of the Laboratory of Biological Control of the UFV and monitored separately. Hatched nymphs were kept in Petri dishes ( $13.0 \times 1.5$  cm) with moistened cotton wool and fed with *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) pupae until the nymphs transformed into the adult stage. Water was supplied in 5 mL anesthetic tubes plugged with moistened cotton wool. Egg masses deposited in the cages were collected with cotton wool and transferred to Petri dishes ( $9.0 \times 1.5$  cm) with moistened cotton wool. Nymphs were maintained in these dishes and fed *T. molitor* pupae.

##### Choice of Sexual Partner

Newly emerged *P. nigrispinus* females were transferred to plastic containers (500 mL) and fed every 2 days with 2 *T. molitor* pupae. Treatments were: 1) one virgin female with one virgin sibling male, 2) one virgin female with one virgin non-sibling male and 3) one virgin female simultaneously with 2 virgin males (a sibling and a non-sibling male). Sibling males were marked on the back

(scutellum) with water-based ink. Females of the no-choice tests were kept in containers either with a single sibling or non-sibling male. In the choice test, each female was kept with one sibling and one non-sibling male. Ten replications were used per treatment (Oliver & Cordero 2009). The weight of the females used ranged from 70-80 mg and that of males from 43-50 mg (Torres & Zanuncio 2001).

##### Data Collection

Number of matings, eggs and egg masses were observed every 12 h from the time adults were placed in the containers. Eggs laid were removed from the plastic containers with cotton tips and transferred to Petri dishes ( $9.5 \times 1.5$  cm) containing moistened cotton wool to prevent dehydration. Egg viability was evaluated during the first 10 days after each oviposition. Longevities of females with sibling, non-sibling or both males were recorded.

##### Statistical Analysis

The number of matings of females without a choice of male was subjected to analysis of variance (ANOVA) ( $P < 0.05$ ). A chi-square test was applied to the data from females with a choice of partners. The numbers of eggs, eggs/egg mass, egg masses, nymphs and duration of oviposition period and female longevity were analyzed with the non-parametric Kruskal-Wallis test.

#### RESULTS

In the no-choice tests, the numbers of matings were similar for females mated with siblings or non-siblings males (Fig. 1A) ( $3.3 \pm 1.36$ , and  $1.7 \pm 0.73$  times, respectively;  $\chi^2 = 2.0$ ;  $P > 0.05$ ).

The average oviposition period of a female mated both with a sibling + a non-sibling male was shorter (6-7 days) than the oviposition periods following a mating with either a sibling or a non-sibling male (16-16.5 days) (Fig. 1B). Number of eggs per female was the lowest when the female had mated with a sibling + a non-sibling male (choice) (Fig. 2A). However, numbers of eggs/egg mass and of egg masses did not differ between females mated with siblings, non-siblings or with both types of males (Fig. 2B, C).

The average number of nymphs per female that had mated with a non-sibling male was the highest, the number of nymphs per female that had mated with a sibling were intermediate and the number of nymphs per female that had mated with both kinds of males produced the fewest nymphs (Fig. 3A). The type of male with which the female mated did not affect female longevity (Fig. 3B).

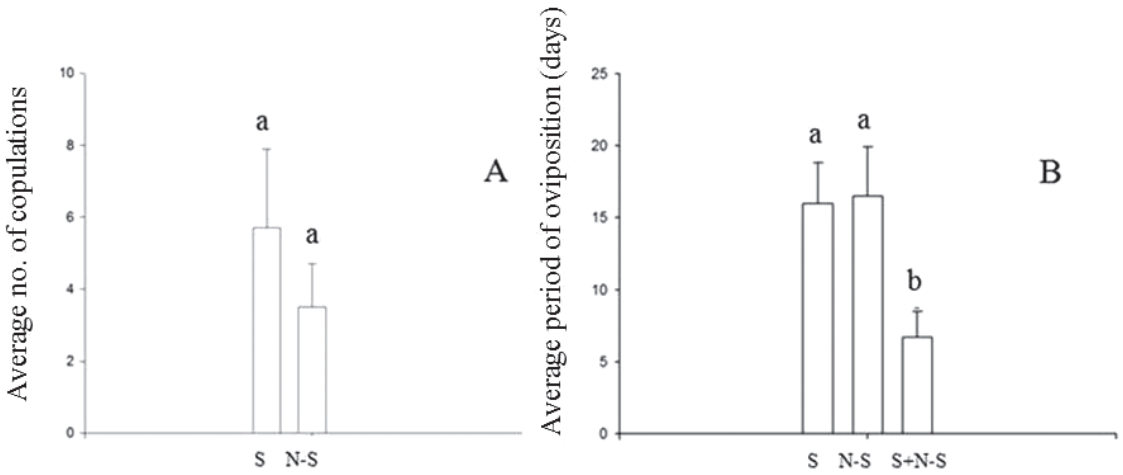


Fig. 1. Number of matings (A) (ANOVA:  $F_{1,18} = 0.7458$ ;  $P > 0.05$ ) and oviposition periods (days) (B) (Kruskal-Wallis test:  $KW = 8.2518$ ;  $P < 0.05$ ) of *Podisus nigrispinus* females each held either with a sibling, a non-sibling or both kinds of males in the laboratory at  $28 \pm 1$  °C,  $70 \pm 10\%$  RH and 12:12 h L:D. Bars on the columns represent standard errors. Abbreviations: S - sibling; N-S - non-sibling; and S + N-S - sibling plus non-sibling.

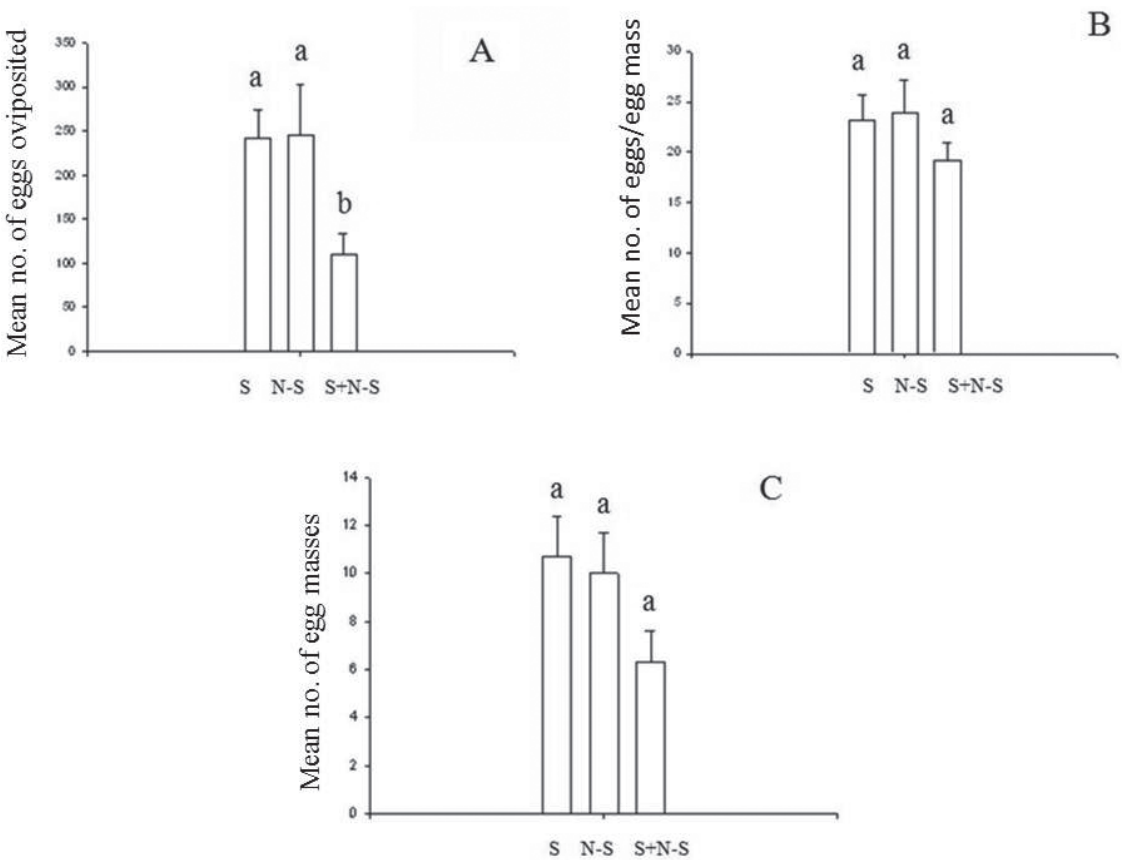


Fig. 2. Number of eggs oviposited (A) (Kruskal-Wallis test:  $KW = 6.0351$ ;  $P < 0.05$ ); number of eggs/egg mass (B) (Kruskal-Wallis test:  $KW = 3.2535$ ;  $P > 0.05$ ); and number of egg masses (C) (Kruskal-Wallis test:  $KW = 1.9766$ ;  $P > 0.05$ ) per *Podisus nigrispinus* female held either with sibling, non-sibling or both kinds of males in the laboratory at  $28 \pm 1$  °C,  $70 \pm 10\%$  RH and 12:12 h L:D. Bars on the columns represent standard errors. Abbreviations: S - sibling; N-S - non-sibling; and S + N-S - sibling plus non-sibling.

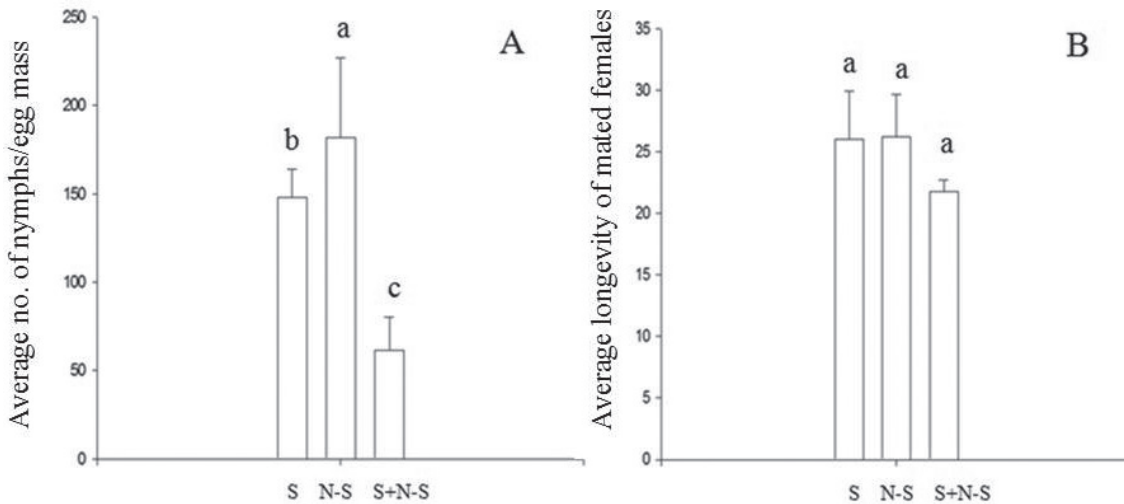


Fig. 3. Mean number of nymphs per egg mass (A) (Kruskal-Wallis test:  $KW = 6.1170$ ;  $P < 0.05$ ) and mean longevities (B) (Kruskal-Wallis test:  $KW = 1.0721$ ;  $P > 0.05$ ) of *Podisus nigrispinus* females each held either with a sibling, a non-sibling or both kinds males in the laboratory at  $28 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH and 12:12 h L:D. Bars on columns represent standard errors. Abbreviations: S - sibling; N-S - non-sibling; and S + N-S - sibling plus non-sibling.

#### DISCUSSION

Females of *P. nigrispinus* did not discriminate between sibling and non-sibling males in the laboratory. This may lead to decline of fitness due to inbreeding as observed for many insect species (Henter 2003; Clutton-Brock 2007; Shuker 2010).

The shorter oviposition period of females in the choice-test may reflect conflict between males, because the number of copulations should have been adequate to fertilize most eggs produced (Torres & Zanuncio 2001). Quantity of sperm transferred during mating can be low and sperm competition high in polygamous insects, which can decrease oviposition periods (Parker et al. 1997; Arnqvist & Nilsson 2000; Elgar et al. 2003; Crudgington et al. 2010; Rodrigues et al. 2008).

The greater number of eggs laid and the higher egg viability of *P. nigrispinus* females mated with non-sibling males indicate that this predator can reduce inbreeding by mating with unrelated males. Inbreeding could be reduced or avoided in laboratory rearing facilities by keeping *P. nigrispinus* females separate from sibling males. Sexual conflict between males and females after mating is ubiquitous and increases competition between the first ones to fertilize eggs (Stockley 1997).

The similar longevities of females in the various treatments may indicate no differences in quantities of sperm and accessory substances transferred by *P. nigrispinus* males. The nutritional content of sperm of this stinkbug has been poorly studied, but males of other insects provide sperm rich in nutrients as a nuptial gift (Pivnick & McNeil 1987; Koshiyama et al. 1996; Smedley

& Eisner 1996). Accessory substances are used in the processes of cellular respiration and vitellogenesis (Koshiyama et al. 1996), oviposition (Herndon & Wolfner 1995), sperm activation (Osanai & Chen 1993) and to maintain sperm viability during storage in the spermatheca (Tram & Wolfner 1999), all of which tend to increase female longevity.

The increased oviposition and egg viability rates for females that had mated with non-sibling males confirm the need of multiple mating for the reproductive success of this species (Torres & Zanuncio 2001). *Podisus nigrispinus* nymphs aggregate from the time of hatching until transformation into adults (Torres & Zanuncio 2001), which increases the chances of mating with siblings and inbreeding.

#### CONCLUSIONS

Females of *P. nigrispinus* did not discriminate between sibling or non-sibling males for mating, which can lead to genetic inbreeding. However, the greater number of eggs laid and the higher egg viability of *P. nigrispinus* females mated with non-sibling males indicate that this predator can reduce genetic inbreeding by mating with genetically unrelated males.

#### ACKNOWLEDGMENTS

To the Brazilian agencies "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)" and "Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)" for financial support.

## REFERENCES CITED

- ARNQVIST, G., AND NILSSON, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behav.* 60: 145-164.
- CHAPMAN, T., LIDDLE, L. F., KALB, J. M., WOLFNER, M. F., AND PARTRIDGE, L. (1995) Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* 373: 241-244.
- CLUTTON-BROCK, T. 2007. Sexual selection in males and females. *Science* 318: 1882-1885.
- CRUDGINGTON, H. S., AND SIVA-JOTHY, M. T. 2000. Genital damage, kicking and early death. *Nature* 407: 855-856.
- CRUDGINGTON, H. S., FELLOWS, S., AND SNOOK R. R. 2010. Increased opportunity for sexual conflict promotes harmful males with elevated courtship frequencies. *J. Evol. Biol.* 23: 440-446.
- ELGAR, M. A., CRESPIGNY, F. E. C., AND RAMAMURTHY, S. 2003. Male copulation behaviour and risk of sperm competition. *Animal Behav.* 66: 211-216.
- HENTER, H. J. 2003. Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. *Evolution* 57: 1793-1803.
- HERNDON, L. A., AND WOLFNER, M. F. 1995. A drosophila seminal fluid protein, Acp26Aa, stimulates egg laying in females for 1 day after mating. *Proc. Natl. Acad. Sci. USA* 92: 10114-10118.
- KOSHIYAMA, Y., TSUMUKI, H., FUJISAKI, K., AND NAKASUJI, F. 1996. Nutritional contribution to females of <sup>14</sup>C-labeled male secretions transferred during mating in *Menida scotti* (Heteroptera: Pentatomidae). *Res. Popul. Ecol.* 38: 51-56.
- MEDEIROS, R. S., RAMALHO, F. S., ZANUNCIO, J. C., AND SERRÃO, J. E. 2003. Effect of temperature on life table parameters of *Podisus nigrispinus* (Heteroptera: Pentatomidae) fed with *Alabama argillacea* (Lepidoptera: Noctuidae) larvae. *J. Appl. Entomol.* 127: 209-213.
- MOLINA-RUGAMA, A. J., ZANUNCIO, J. C., TORRES, J. B., AND ZANUNCIO, T. V. 1997. Longevity and fecundity of *Podisus nigrispinus* (Heteroptera: Pentatomidae) fed *Musca domestica* (Diptera: Muscidae) and bean. *Rev. Biol. Trop.* 45: 1125-1130.
- OLIVER, C., AND CORDERO, C. 2009. Multiple mating reduces male survivorship but not ejaculate size in the polygamous insect *Stenomacra marginella* (Heteroptera: Largidae). *Evol. Ecol.* 23: 417-424.
- OSANAI, M., AND CHEN, P. S. 1993. A comparative study on the arginine degradation cascade for sperm maturation of *Bombyx mori* and *Drosophila melanogaster*. *Amino Acids* 5: 341-350.
- PARKER, G. A., BALL, M. A., STOCKLEY, P., AND GAGE, M. J. G. 1997. Sperm competition games: a prospective analysis of risk assessment. *Proc. R. Soc. B: Biol. Sci.* 264: 1793-1802.
- PIVNICK, K. A., AND MCNEIL, J. N. 1987. Puddling in butterflies: sodium affects reproductive success in *Thymelicus lineola*. *Physiol. Entomol.* 12:461-472.
- REYNOLDS, J. D. 1996. Animal breeding systems. *Trends Ecol. Evol.* 11: 68-72.
- RIDLEY, M. 1990. The control and frequency of mating in insects. *Funct. Ecol.* 4: 75-84.
- RODRIGUES, A. R. S., SERRÃO, J. E., TEIXEIRA, V. W., TORRES, J. B., AND TEIXEIRA, A. A. 2008. Spermatogenesis, changes in reproductive structures, and time constraint associated with insemination in *Podisus nigrispinus*. *J. Insect Physiol.* 54: 1543-1551.
- RODRIGUES, A. R. S., TORRES, J. B., SIQUEIRA, H. A. A., AND TEIXEIRA, V. W. 2009. *Podisus nigrispinus* requer cópulas longas para o sucesso reprodutivo. *Neotrop. Entomol.* 38: 746-753.
- ROLFF, J., AND SIVA-JOTHY, M. T. 2002. Copulation corrupts immunity: a mechanism for a cost of mating in insects. *Proc. Natl. Acad. Sci. USA* 99: 9916-9918.
- SHUKER, D. M. 2010. Sexual selection: endless forms or tangled bank? *Animal Behav.* 79: E11-E17.
- SMEDLEY, S. R., AND EISNER, T. 1996. Sodium: a male moth's gift to its offspring. *Proc. Natl. Acad. Sci. USA* 93: 809-813.
- SOUSA-SOUTO, L., EVANGELISTA JR, W. S., LIMA, E. R., ZANUNCIO, J. C., AND FONSECA, M. G. 2006. Sperm depletion: a cost for single mated females of *Podisus nigrispinus* (Dallas, 1851) (Heteroptera: Pentatomidae). *Brazilian Arch. Biol. Technol.* 49: 923-926.
- STOCKLEY, P. 1997. Sexual conflict resulting from adaptations to sperm competition. *Trends Ecol. Evol.* 12: 154-159.
- TIPPING, P. W., HOLKO, C. A., ABDUL-BAKI, A. A., AND ALDRICH, J. R. 1999. Evaluating *Edovum puttleri* Grissell and *Podisus maculiventris* (Say) for augmentative biological control of Colorado potato beetle in tomatoes. *Biol. Control* 16: 35-42.
- TORRES, J. B., AND ZANUNCIO, J. C. 2001. Effects of sequential mating by males on reproductive output of the stinkbug predator, *Podisus nigrispinus*. *BioControl* 46: 469-480.
- TRAM, U., AND WOLFNER, M. F. 1999. Male seminal fluid proteins are essential for sperm storage in *Drosophila melanogaster*. *Genetics* 153: 837-844.
- WATSON, P. J., ARNQVIST, G., AND STALLMANN, R. R. 1998. Sexual conflict and the energetic costs of mating and mate choice in water striders. *American Nat.* 151: 46-58.
- ZANUNCIO, J. C., MOLINA-RUGAMA, A. J., SERRÃO, J., AND PRATISSOLI, D. 2001. Nymphal development and reproduction of *Podisus nigrispinus* (Heteroptera: Pentatomidae) fed with combinations of *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae and *Musca domestica* (Diptera: Muscidae) larvae. *Biocontrol Sci. Technol.* 11: 331-337.