Ideal period for mating females of the predator

*Podisus fuscescens* (Dallas, 1851) (Hemiptera: Pentatomidae)

Periodo ideal para el apareamiento de hembras del depredador *Podisus fuscescens* (Dallas, 1851) (Hemiptera: Pentatomidae)

**Abstract:** The improvement of techniques for the mass rearing of predators in the laboratory, including the age of mating is important to obtain the largest number of individuals for biological control programs. The purpose of this study was to evaluate the females’ fecundity of the predator *Podisus fuscescens* (Dallas, 1851) (Hemiptera: Pentatomidae) with different pre-mating periods. The females of this predator were mated immediately after emergence (T1) or with one (T2), two (T3), three (T4), four (T5) or five (T6) days after emergence. The females mated immediately and one day after the emergency (T1 and T2) had fewer egg clusters than those mated after five days (T6). The number of nymphs per female was lower for those mated immediately after the emergence (T1 and one (T2) or two days (T3). The total number of eggs was lower in females mated one day after emergence (T2) than for those mated after five days (T6). The proportion of hatched eggs was lower in the treatments T1 and T2 than in the T6. The oviposition and longevity of *P. fuscescens* females were similar between treatments; however, the pre-oviposition period longer in the T1 than T5 and T6. The pos-oviposition period was longer in the T1. *Podisus fuscescens* females require five days after emergence to mature their reproductive system, which improves their fitness and avoids unnecessary mating.

**Keywords:** Asopinae, biological control, mass rearing, reproduction.
Introduction

The family Pentatomidae comprises important predators responsible for the biological control of Lepidoptera and Coleoptera larvae (Soares et al. 2009; De Carvalho et al. 2020; Pires et al. 2020; Cornelius et al. 2021). However, phytophagous species of this order are pests of wild and cultivated plants (Soares et al. 2022). *Podisus* is the most diverse genus in the Pentatomidae, with species distributed in the Neotropical and Nearctic regions (Brugnera et al. 2020; Oliveira-Júnior et al. 2021; Roell 2021).

The predatory stink bug *Podisus fuscescens* (Dallas, 1851) (Hemiptera: Pentatomidae) (often identified in the scientific literature as *P. distinctus* (Stål) in the last two decades) has generalist predatory habits and survives in the field, even when its prey populations is at low level (Lacerda et al. 2004; Brugnera et al. 2020). The efficiency of this natural enemy depends on its high ability to reproduce, characterizing it as an r strategist, with high oocyte development, ovulation and oviposition rates (Force 1972). Furthermore, mating and components of the ejaculation increases female reproductive output in many insect species (Nanfack-Minkeu and Sirot 2022).

Polyandry, multiple mating by females, increases the genetic variability in insects (Dahan et al. 2022). Females of *Podisus* spp. are polyandrous and can copulate in the laboratory up to 13 times during its reproductive cycle. However, three to four mating are sufficient to fertilize the eggs and to maintain the nymph hatching above 80% during the female lifetime (Torres et al. 1997; Soares et al. 2011). The period necessary for the reproductive apparatus to mature is also important to maintain the females’ fertility in the laboratory. Ovaries of the predator *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae) are immature when they emerge, but after seven days, their ovaries are developed with stored nutritive cells known as guard cells or trophocytes, which are necessary for the reproductive apparatus to mature is also necessary to maintain the females’ fertility in the laboratory. Ovaries of the predator *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae) are immature when they emerge, but after seven days, their ovaries are developed with stored mature oocytes (Soares et al. 2011). The period for the maturation of the ovaries of *P. fuscescens* is unknown.

Understanding the reproduction of *P. fuscescens* and determining the ideal mating age can improve the mass rearing techniques of this predator. Avoiding unnecessary copulations prevents the insects from wasting energy or becoming injured, reducing the individuals’ longevity. The objective of this work was to evaluate the fecundity of females of the predator *P. fuscescens* with different pre-mating periods.

Materials and methods

The experiment was carried out in the laboratory at an air temperature of 25 ± 2 °C, an air relative humidity of 65 ± 10% and a photophase of 12 h. Individuals of *P. fuscescens* were immature when they emerged, but after seven days, their ovaries are developed with stored nutritive cells known as guard cells or trophocytes, which are necessary for the reproductive apparatus to mature is also necessary to maintain the females’ fertility in the laboratory. Ovaries of the predator *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae) are immature when they emerge, but after seven days, their ovaries are developed with stored mature oocytes (Soares et al. 2011). The period for the maturation of the ovaries of *P. fuscescens* is unknown.

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The experiment was carried out in the laboratory at an air temperature of 25 ± 2 °C, an air relative humidity of 65 ± 10% and a photophase of 12 h. Individuals of *P. fuscescens* were obtained from mass rearing at the Laboratory for Biological Control of Insects (LCBio) of the Universidade Federal de Viçosa. Three hundred eggs from different egg masses were collected and separated in Petri dishes (9.0 x 1.5 cm) with a moistened cotton swab until the nymphs hatched. Nymphs were reared in Petri dishes (9.0 x 1.5 cm), in groups of 10 individuals, with a cotton swab moistened with distilled water and with *Tenebrion molitor* L. (Coleoptera: Tenebrionidae) pupae were fed “ad libitum” until adulthood (Soares et al. 2009; 2011).

Females were mated at the day of emergence (T1) or at one (T2), two (T3), three (T4), four (T5), or five (T6) days after the adult’s emergence. Males were selected at three days of emergence in all treatments, as they are receptive to copulation from that age onwards (Carvalho et al. 1994).

This homogenization of the age of the male individuals is important to standardize their fertility and the quantity and quality of seminal material transferred to the females. The males were put together with the females for 24 hours (Souza-Souto et al. 2006; Reis et al. 2018) in 500 mL plastic containers, closed with a screened plastic lid to facilitate ventilation. Distilled water was supplied in tubes (anesthetic type) fitted into a hole in the top of the lid.

The eggs were collected and counted daily and were kept in Petri dishes (9.0 X 1.5 cm), with a moistened cotton swab, at 25 ± 2°C and 65 ± 10 % RH. The number of hatched nymphs, eggs, eggs laid per female, percentage of eggs hatched, longevity, pre-oviposition, oviposition and post-oviposition periods (days) of the females were evaluated. The experimental design was completely randomized, with six treatments and 15 replications, with each couple of *P. fuscescens* considered one replication. Data were not normally distributed and were analyzed by the non-parametric Kruskal-Wallis test, and the means compared using the Student-Newman-Keuls (SNK) method (P ≤ 0.01).

Results

The number of hatched *P. fuscescens* nymphs was higher in treatment T6 (105.13 ± 29.20) than in treatments T1 (3.60 ± 2.57) and T2 (10.87 ± 8.30) (H = 82.38; P < 0.001) (Fig. 1A).

The total number of eggs per female was lower in the T2 individuals (128.27 ± 32.16) than in the T6 individuals (307.8 ± 4.89) (H = 80.39; P < 0.001) (Fig. 1B). The number of egg masses per female was higher in T6 (14.80 ± 1.74) and lower in T1 (6.4 ± 1.01) and T2 (7.4 ± 1.74) (H = 79.25; P < 0.001) (Fig. 1C).

The number of hatched eggs was lower for couples with females of *P. fuscescens* mated on the emergence day (T1) (8.89 ± 6.88), with one day (T2) (9.91 ± 5.06) or two days (T3) (14.39 ± 7.40) after emergence, than for females at five days after emergence (T6) (51.48 ± 9.58) (H = 73.68; P < 0.001) (Fig. 1D).

The pre-oviposition period was longer for couples with females from treatment T1 (22.80 ± 1.61) than for couples with T5 (13.07 ± 1.84) and T6 (11.07 ± 1.17) (H = 78.56; P < 0.001) (Fig. 1E). However, the oviposition period was similar between treatments, ranging from 29.00 ± 6.47 to 39.73 ± 4.78 (H = 14.73; P = 0.0115) (Fig. 1F). The post-oviposition period was longer for T1 females (13.86 ± 3.92) (H = 68.89; P < 0.001) (Fig. 1G). Longevity was similar among treatments, ranging from 60.33 ± 5.72 to 69.93 ± 6.69 days (H = 9.30; P = 0.097) (Fig. 1H).

Discussion

The higher number of nymphs hatched in the T6 treatment shows that females of the species *P. fuscescens* need approximately five days to mature their reproductive system. This has previously been reported for other species of the genus Podisus. The predator *P. nigrispinus* presents immature ovaries at emergence and, after seven days, mature ovaries, evidenced by the presence of oocytes (Soares et al. 2011). The ovaries of *Podisus maculiventris* (Say, 1832) (Hemiptera: Pentatomidae) are fully matured up to 21 days after the emergence of its female (De Clercq and Degheele 1997). Predatory stink bugs have meristic-type ovaries (Lemos et al. 2005), with nutritive cells known as guard cells or trophocytes, which
supply the oocytes with metabolites and organelles through cytoplasmic bridges. Insects with this ovary type need some days to complete vitellogenesis, unlike insects such as parasitoids of the order Hymenoptera, which emerge with mature oocytes (Jervis et al. 2001). Vitellogenesis is an important control point in oogenesis that integrates hormonal cues to match female physiological conditions (i.e., nutrition, mating status, etc.) (Zhang et al. 2022).

In addition, females of Podisus sp. will have their spermatheca filled with the sperm during copulation. This organ is responsible for storing and maintaining viable sperm for later fertilization (Rodrigues et al. 2008). However, the low

Figure 1. Number of nymphs/female (A), number of eggs/female (B), number of posture/female (C), proportion of hatched posture (%) (D), pre-oviposition period (days) (E), oviposition period (days) (F), Post-oviposition period (days) (G) e longevity (days) (H) of females of Podisus fuscescens (Hemiptera: Pentatomidae), mated immediately after emergence (T1) and with one (T2), two (T3), three (T4), four (T5) and five (T6) days after emergence. Means followed by the same letter do not differ by Kruskal-Wallis test, and the means were compared using the Student-Newman-Keuls (SNK) method (P ≤ 0.01).
viability and absence of fertile eggs in some situations may be due to insufficient transfer of viable sperm (Souza-Souto et al. 2006). Sperm and other ejaculate components are often limited by quantity, quality, and timing of production (Perry et al., 2013; Jarrige et al. 2016). Thus, the species’ reproductive rate increases with mating success (Nason and Kelly 2020). Females of *P. fuscescens* in non-fertile age, at the emergence and up to four days after, could reject copulation. Insects perceive and integrate a hierarchy of visual, chemical, and tactile cues for reproductive purposes (Benelli and Lucchi 2021), even while in confinement. Courtship with males can result in important energy losses, increased injuries and predation risk.

The larger oviposition and offspring yield of *P. fuscescens* females in the treatment T6 can be explained by the stimulation caused by copulation. This hypothesis is reinforced by observing *P. nigrispinus* females, which start egg production and maturation soon after emergence, whether they are mated or not. However, mating is important to maintain this predator’s egg production and oviposition rates (Soares et al. 2011).

The lower offspring production in the treatments T1, T2 and T3 can also be due to the *P. fuscescens* females not being able to produce the necessary secretions to maintain the viability of the sperm in their spermatheca. Such secretions are produced in an accessory gland of the spermatheca (Rodrigues et al. 2008). This gland may not be completely mature before the fifth day after the female emergence. Thus, the increased number of egg masses, eggs, the proportion of hatched eggs and the number of nymphs for females of treatment T6 suggests that five days after the emergence of the female is the ideal period necessary before the initial contact between the sex partners. The sexual maturation of this predator is a progressive process that, until completed, will not allow the females to reach their maximum fertility potential (Carvalho et al. 1994).

The oviposition period and longevity did not differ among treatments. However, the values observed in this work were higher than those of *P. fuscescens* females (reported as *P. distinctus*) mated throughout their life cycle (Pires et al. 2009). The uninterrupted contact between sexual partners, with successive copulations, and the physiological costs of this habit (Vellnow et al. 2020), may have caused the reduction of the reproductive cycle and the premature death of the females in the work of Pires et al. (2009). The longer post-oviposition period in the treatment T1 may be due to lower physiological stress, given the smaller egg masses produced during its reproductive cycle.

The production of many offspring is essential when planning the mass rearing of predators in the laboratory. It also plays an important role in population growth in the field, increasing the survival rate and the probability of a greater number of these insects reaching the adult stage (Lacerda et al. 2004). After emergence in the laboratory, females of *P. fuscescens* must be kept separated from males for five days before being mated and released into the field. For those females intended to maintain mass rearing in the laboratory, mating should also occur only from the fifth day of emergence, allowing for the perfect maturation of the reproductive apparatus. Hence, the control of the ideal mating age and the presence of males in the laboratory mass rearing can benefit the fitness of *P. fuscescens* females and the biological control programs using this predator.

Conclusions

The improvement of the reproductive parameters of *P. fuscescens* females mated at five days after the emergence, evidenced by an increased number of egg masses, eggs, the proportion of hatched eggs and the number of nymphs, indicate that the sexual maturation of *P. fuscescens* is progressive. The sexual contact between partners before this period does not bring benefits for the fitness of this predator.

Literature cited

BENELLI, G.; LUCCHI, A. 2021. Insect pheromones to mating disruption: theory and practice. Insects 12 (8): 698. https://doi.org/10.3390/insects12080698

BRUGNERA, R.; ROELL, T.; CAMPOS, L. A.; GRAZIA, J. 2020. Taxonomy of widespread Neotropical species of *Podisus Herrich-Schäffer* (Hemiptera: Pentatomidae: Asopinae): redescriptions of *P. distinctus* (Stål, 1860) and revalidation of *P. fuscescens* (Dallas, 1851). Zootaxa 4751 (3): 546-562. https://www.biotaxa.org/Zootaxa/article/view/zootaxa.4751.3.7

CARVALHO, R. D. S.; VILELA, E. F.; BORGES, M.; ZANUNCIO, J. C. 1994. Ritmo do comportamento de acasalamento e atividade sexual de *Podisus connexus* Bergroth (Heteroptera: Pentatomidae: Asopinae). Anais da Sociedade Entomológica do Brasil, 23 (2): 197-202. https://doi.org/10.37486/0301-8059.v23i3.929

CORNELIUS, M. L.; HERLIHY, M. V.; VINYARD, B. T.; WEBER, D. C.; GREENSTONE, M. H. 2021. Parasitism and predation on sentinel egg masses of three stink bug species (Hemiptera: Pentatomidae) in native and exotic ornamental landscapes. Journal of Economic Entomology 114 (2): 590-596. https://doi.org/10.1093/jee/toaa329

DAHAN, R. A.; GROVEI, N. K.; BOLLAZZI, M.; GERSTNER, B. P.; RABELING, C. 2022. Decoupled evolution of mating biology and social organization in *Acromyrmex* leaf-cutting ants. Behavioral Ecology and Sociobiology 76: 7. https://doi.org/10.1007/s00265-021-03113-1

DE CARVALHO, J. R.; PRATISGOLLI, D.; DE ARAÚJO JUNIOR, L. M.; DAMASCENA, A. P.; HOLTZ, A. M.; DALVI, L. P.; VIANNA, U. R. 2020. Predation behavior of *Podisus nigrispinus* on *Spodoptera eridania*. Journal of Asia-Pacific Entomology 23 (4): 1279-1282. https://doi.org/10.1016/j.aspen.2020.10.007

DE CLERCQ, P.; DEGHEELE, D. 1997. Effects of mating status on body weight, oviposition, egg load, and predation in the predatory stinkbug *Podisus maculiventris* (Heteroptera: Pentatomidae). Annals of the Entomological Society of America 90 (2): 121-127. https://doi.org/10.1093/aesa/90.2.121

FORCE, D. C. 1972. R and K-strategists in endemic host-parasitoid communities. Bulletin of the ESA 18(3): 135-137. https://doi.org/10.1093/besa/18.3.135

JARRIGE, A.; KASSIS, A.; SCHMOLL, T.; GOUBAULT, M. 2016. Recently mated males of a lek-mating insect intensify precopulatory mate guarding under male competition. Animal Behaviour 117: 21-34. https://doi.org/10.1016/j.anbehav.2016.04.012

JERVIS, M. A.; HEIMPEL, G. E.; FERNS, P. N.; HARVEY, J. A.; KIDD, N. A. C. 2001. Life-history strategies in parasitoid wasps: a comparative analysis of “ovigeny”. Journal of Animal Ecology 70 (3): 442-458. https://doi.org/10.1046/j.1365-2656.2001.00507.x

LACERDA, M. C.; FERREIRA, A. M. R. M.; ZANUNCIO, T. V.; ZANUNCIO, J. C.; BERNARDINO, A. S.; ESPINDULA M. C. 2004. Development and reproduction of *Podisus distinctus* (Heteroptera: Pentatomidae) fed on larvae of *Bombyx mori* (Lepidoptera: Bombycidae). Revista Brasileira de Biologia 64 (2): 1365-2656.2001.00507.x

LACERDA, M. C.; FERREIRA, A. M. R. M.; ZANUNCIO, T. V.; ZANUNCIO, J. C.; BERNARDINO, A. S.; ESPINDULA M. C. 2004. Development and reproduction of *Podisus distinctus* (Heteroptera: Pentatomidae) fed on larvae of *Bombyx mori* (Lepidoptera: Bombycidae). Revista Brasileira de Biologia 64 (2): 1365-2656.2001.00507.x
fed on different diets. Brazilian Archives of Biology and Technology 48 (1): 129-138. https://doi.org/10.1590/S1516-89132005000100017

NANFACK-MINKEU, F.; SIROT, L. K. 2022. Effects of mating on gene expression in female insects: unifying the field. Insects 13 (1): 69. https://doi.org/10.3390/insects13010069

NASON, S. E.; KELLY, C. D. 2020. Benefits of multiple mating in a sexually dimorphic polygynandrous insect. Animal Behaviour 164: 65-72. https://doi.org/10.1016/j.anbehav.2020.03.018

OLIVEIRA-JUNIOR, G. S.; OLIVEIRA, D. K. S.; VIEIRA, E. R. D.; SOUZA, M. W. R.; SANTOS, M. M.; ASSIS-JUNIOR, S. L.; SOARES, M. A. 2021. First record of the predatory stink bug Podisus sagitta (Hemiptera: Asopinae) in Brazil. Brazilian Journal of Biology 82: e236863. https://doi.org/10.1590/1519-6984.236863

PERRY, J. C.; SIROT, L.; WIGBY, S. 2013. The seminal symphony: how to compose an ejaculate. Trends in Ecology & Evolution 28 (7): 414-422. https://doi.org/10.1016/j.tree.2013.03.005

PIRES, E. M.; ZANUNCIO, J. C.; NOGUERA, R. M.; SOARES, M. A.; DE OLIVEIRA, M. A. 2020. Dispersal of the zoophytophagous predator Brontocoris tabidus and Podisus nigrispinus (Heteroptera: Pentatomidae) in an eucalyptus plantation. Florida Entomologist 103 (2): 168-171. https://doi.org/10.1653/024.103.0203

PIRES, E. M.; AZEVEDO, D. O.; LIMA, E. R.; PINTO, R.; PELUZIO, R. J. E.; SERRÃO, J. E.; ZANUNCIO, J. C. 2009. Development, reproduction and performance predatory of percevejo zoofitófago Podisus distinctus (Hemiptera: Pentatomidae) alimentado com larvas de Musca domestica (Diptera: Muscidae) imobilizadas ou soltas. Revista Brasileira de Biociências 7 (3): 280-284. http://www.ufg.rs.br/scerbio/ols/index.php/rbb/article/view/1204

REIS, T. C.; SOARES, M. A.; SANTOS, J. B.; SANTOS, C. A.; SERRAJO, J. E.; ZANUNCIO, J. C.; FERREIRA, E. A. 2018. Atrazine and nicosulfuron affect the reproductive fitness of the predator Podisus nigrispinus (Hemiptera: Pentatomidae). Anais da Academia Brasileira de Ciências 90 (4): 3625-3633. https://doi.org/10.1590/0001-3765201820170748

RODRIGUES, A. R. S.; SERRÃO, J. E.; TEIXEIRA, W. W.; TORRES, J. B.; TEIXEIRA, A. A. 2008. Spermatogenesis, changes in reproductive structures, and time constraint associated with insemination in Podisus nigrispinus. Journal of Insect Physiology 54 (12): 1543-1551. https://doi.org/10.1016/j.jinsphys.2008.09.003

ROELL, T.; BRUGNERA, R.; LEMAITRE, V. A. 2021. Lost and found-Discovery of the presumed lost type of Arma pallipes Dallas, new synonymy and description of two new species of Podisus Herrich-Schaffer (Hemiptera: Pentatomidae: Asopinae). Zootaxa, 4958 (1): 1-14. https://doi.org/10.1164/zootaxa.4958.1.33

SOARES, M. A.; FAUSTINO-JÚNIOR, W.; OLIVEIRA, D. K. S.; CALDEIRA, Z. V.; SILVA, E. T. L.; ASSIS-JÚNIOR, S. L.; ZANUNCIO, J. C.; ZANUNCIO, A. J. V. 2022. Edessa rajiformarginata (Hemiptera: Pentatomidae) feeding on Solanum lycocarpum (Solanaceae) in rupetean fields of the Brazilian Cerrado biome. Brazilian Journal of Biology 82: e235839. https://doi.org/10.1590/1519-6984.235839

SOARES, M. A.; BATISTA, J. D.; ZANUNCIO, J. C.; LINENETO, J.; SERRÃO, J. E. 2011. Ovary development, egg production and oviposition for mated and virgin females of the predator Podisus nigrispinus (Heteroptera: Pentatomidae). Acta Scientiarum Agronomy 33 (4): 597-602. https://doi.org/10.4025/actasciagon.v33i4.6694

SOARES, M. A.; ZANUNCIO, J. C.; LEITE, G. L. D.; WERME-LINGER, E. D.; SERRÃO, J. E. 2009. Does Thyrinteina arnobia (Lepidoptera: Geometridae) use different defense behaviors against predators? Journal of Plant Diseases and Protection 116: 30-33. https://doi.org/10.1007/BF03356283

SOUSA-SOUTO, L.; EVANGELISTA-JR, W. S.; LIMA, E. R.; ZANUNCIO, J. C.; FONSECA, M. G. 2006. Sperm depletion: a cost for single mated females of Podisus nigrispinus (Dallas, 1851) (Heteroptera: Pentatomidae). Brazilian Archives of Biology and Technology 49 (6): 923-926. https://doi.org/10.1590/S1516-89132006000700009

TORRES, J. B.; ZANUNCIO, J. C.; OLIVEIRA, M. C. 1997. Matting frequency and its effect on female reproductive output in the stinkbug predator Podisus nigrispinus (Heteroptera: Pentatomidae). Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 62 (2b): 491-498.

VELLNOW, N.; SCHINDLER, S.; SCHMOLL, T. 2020. Genotype-by-environment interactions for precopulatory mate guarding in a lek-mating insect. Ecology and Evolution 10 (21): 12138-12146. https://doi.org/10.1002/ece3.6841

ZHANG, C.; KIM, A. J.; RIVERA-PEREZ, C.; NORIEGA, F. G.; KIM, Y. J. 2022. The insect somatostatin pathway gates vitellogenesis progression during reproductive maturation and the post-mating response. Nature Communications 13: 969 https://doi.org/10.1038/s41467-022-28592-2

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IMS, VGMS, ARPP, and MAS conceived and designed surveys. MJSC, GLDL, and JCZ contributed analytical tools. IMS, VGMS, MAS and JCZ analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

Conflict interest
The authors who participated in this publication made significant contributions to the manuscript; all authors agree and express that there are no conflicts of interest in this study.