

# Ideal period for mating females of the predator *Podisus fuscescens* (Dallas, 1851) (Hemiptera: Pentatomidae)

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

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**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 with 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.

**Resumen:** Mejorar las técnicas para criar depredadores en el laboratorio, incluida la edad de apareamiento, es importante para obtener el mayor número de individuos para los programas de control biológico. El objetivo de este trabajo fue evaluar la fecundidad de hembras del depredador *Podisus fuscescens* (Dallas, 1851) (Hemiptera: Pentatomidae) con diferentes períodos de pre-apareamiento. Los tratamientos fueron: las hembras de este depredador se aparearon inmediatamente después de la emergencia (T1) o con uno (T2), dos (T3), tres (T4), cuatro (T5) o cinco (T6) días después de la emergencia. Las hembras que se aparearon inmediatamente (T1) y un día después de la emergencia (T2) tuvieron menos posturas que las que se aparearon después de cinco días (T6). El número de ninfas por hembra fue menor para las apareadas en emergencia (T1) y uno o dos días después. El número total de huevos fue menor para las hembras apareadas un día después de la emergencia (T2) que para aquellas apareadas después de cinco días (T6). La proporción de huevos eclosionados fue menor en los tratamientos T1 y T2 en comparación con el tratamiento T6. El período de oviposición y la longevidad de las hembras de *P. fuscescens* fueron similares entre los tratamientos, sin embargo, el período de preoviposición fue más largo para las hembras del tratamiento T1 que para las de T5 y T6 y el período de posoviposición fue más largo en las hembras del tratamiento T1. Las hembras de *P. fuscescens* necesitan cinco días después de la emergencia para que su sistema reproductivo madure, lo que mejora su aptitud y evita copulaciones innecesarias.

**Palabras clave:** Asopinae, control biológico, cría masiva, reproducción.

## 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 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  $25 \pm 2$  °C, an air relative humidity of  $65 \pm 10\%$  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 *Tenebrio 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  $25 \pm 2$  °C and  $65 \pm 10\%$  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 ( $P \leq 0.01$ ).

## Results

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

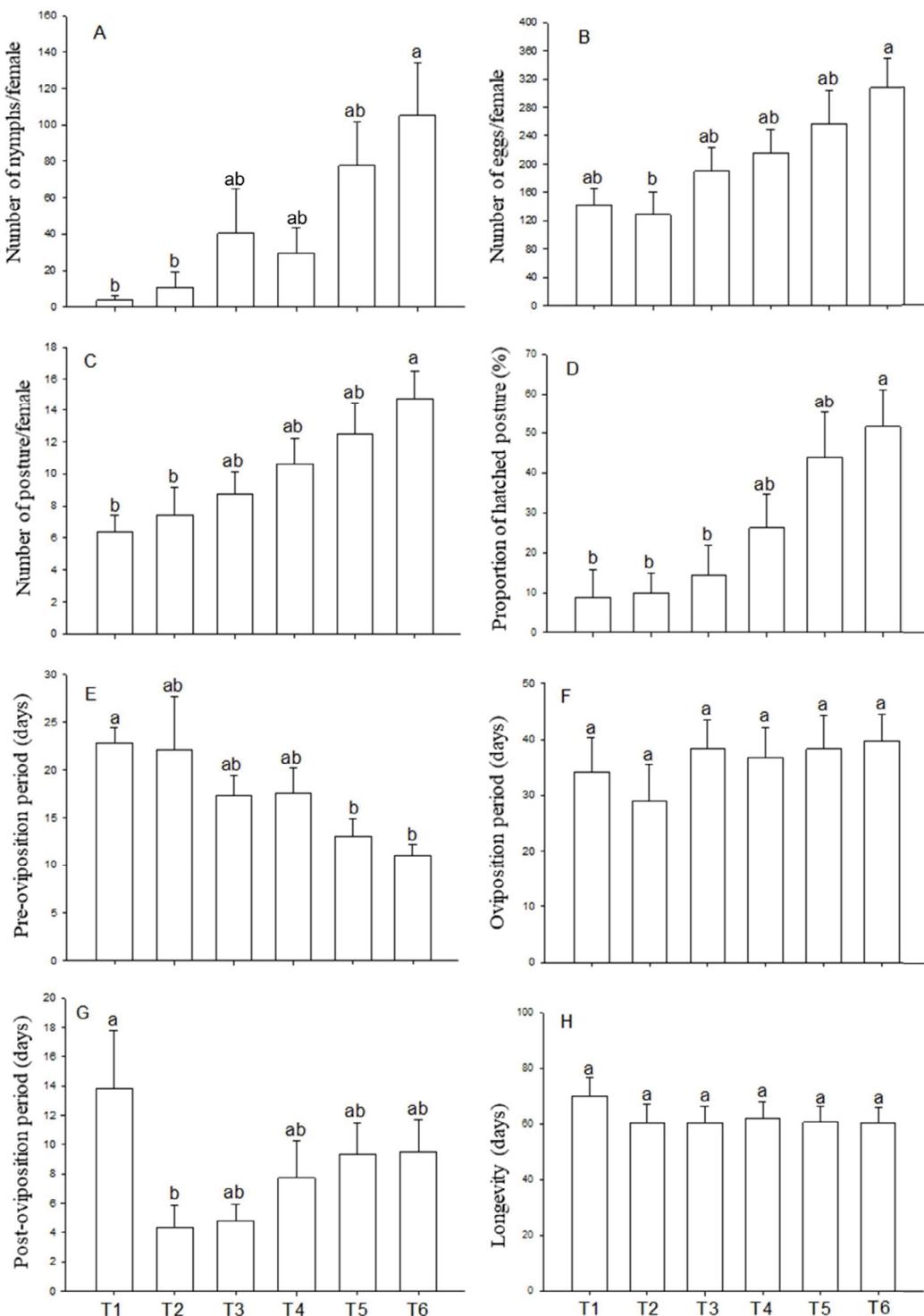
The total number of eggs per female was lower in the T2 individuals ( $128.27 \pm 32.16$ ) than in the T6 individuals ( $307.8 \pm 4.89$ ) ( $H = 80.39$ ;  $P < 0.001$ ) (Fig. 1B). The number of egg masses per female was higher in T6 ( $14.80 \pm 1.74$ ) and lower in T1 ( $6.4 \pm 1.01$ ) and T2 ( $7.4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

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

The pre-oviposition period was longer for couples with females from treatment T1 ( $22.80 \pm 1.61$ ) than for couples with T5 ( $13.07 \pm 1.84$ ) and T6 ( $11.07 \pm 1.17$ ) ( $H = 78.56$ ;  $P < 0.001$ ) (Fig. 1E). However, the oviposition period was similar between treatments, ranging from  $29.00 \pm 6.47$  to  $39.73 \pm 4.78$  ( $H = 14.73$ ;  $P = 0.0115$ ) (Fig. 1F). The post-oviposition period was longer for T1 females ( $13.86 \pm 3.92$ ) ( $H = 68.89$ ;  $P < 0.001$ ) (Fig. 1G). Longevity was similar among treatments, ranging from  $60.33 \pm 5.72$  to  $69.93 \pm 6.69$  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 mature up to 21 days after the emergence of its female (De Clercq and Degheele 1997). Predatory stink bugs have meroistic-type ovaries (Lemos *et al.* 2005), with nutritive cells known as guard cells or trophocytes, which



**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 ( $P \leq 0.01$ ).

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

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.

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## Author contribution

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.