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Sexual behavior of *Anastrepha striata* Schiner (Diptera: Tephritidae) under laboratory conditions

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ABSTRACT

Anastrepha striata Schiner is one of the seven species of tephritidae that are economically important in Brazilian fruit growing, which can infest more than 16 families of host plants, mainly the Myrtaceae. The objective was to obtain information on the age of sexual maturation of males and females and to describe their reproductive behavior in the laboratory. Males reached sexual maturity between 12 and 16 days after emergence, in which most individuals were sexually mature at 14 days of age. During this period, the males performed rapid movements of the wings, producing audible signals. After the females were attracted, the males performed several courtship movements. Females reached sexual maturity between 16 and 19 days after emergence, and the majority was sexually mature at 17 days of age. Daily exhibitions of sexual activity occurred between 5 p.m. and 6 p.m. The species A. striata presented a marked pattern of protandry.

Key words: reproductive behavior, fruit flies, circadian rhythm

Comportamento sexual de Anastrepha striata Schiner (Diptera: Tephritidae) em condições de laboratório

RESUMO

Anastrepha striata Schiner é uma das sete espécies de tephritidae de importância econômica na fruticultura do Brasil, podendo infestar mais de 16 famílias de plantas hospedeiras, principalmente das Mirtáceas. O objetivo foi obter informações sobre a idade de maturação sexual de machos e fêmeas e descrever o seu comportamento reprodutivo em laboratório. Os machos atingiram a maturidade sexual entre 12 e 16 dias após a emergência, com a maioria dos indivíduos sexualmente maduros aos 14 dias de idade. Durante este período, os machos realizaram rápidos movimentos das asas, produzindo sinais audíveis. Após a atração das fêmeas, os machos realizaram diversos movimentos de cortejo. As fêmeas alcançaram a maturidade sexual entre 16 e 19 dias da emergência, com a maioria, sexualmente maduras aos 17 dias de idade. As exibições diárias de atividades sexuais ocorreram entre 17:00 e 18:00h. A espécie *A. striata* apresentou um acentuado padrão de protandria.

Palavras-chave: comportamento reprodutivo, moscas-das-frutas, ritmo circadiano

Introduction

Brazil is one of the world's largest producers of fruit, and considering the production of the 22 main species of Brazilian fruit, it maintains a harvest volume of approximately 40 million tons per year (Beling, 2015). However, the occurrence of several species of fruit flies (Diptera: Tephritidae), distributed throughout the country is a factor that limits production and makes it difficult to export fresh fruit due to the sanctions established by the importing countries regarding the possibility of entry of pests that do not exist in their territories (Facholi-Bendassoli & Uchôa-Fernandes, 2006; Lima et al., 2012).

The species of tephritids existing in Brazil are distributed in four genera: *Anastrepha, Bactrocera*, *Ceratitis* and *Rhagoletis* (Zucchi, 2000a). The genus *Anastrepha* is the most diverse in the country, with 115 species recorded, but only 7 species are economically important: *Anastrepha grandis* (Macquart, 1846), *A. fraterculus* (Wiedemann, 1830), *A. obliqua* (Macquart, 1835), *A. pseudoparallela* (Loew, 1873), *A. sororcula* (Zucchi, 1979), *A. striata* (Schiner, 1868) and *A. zenildae* (Zucchi, 1979) (Zucchi, 2000b; Zucchi, 2016).

The importance of fruit flies is directly related to the damage they cause to fruits, such as rapid maturation, premature fall and rotting of the pulp due to the oviposition of the females and feeding of the larvae on the fruit, besides increasing the necessary costs to control it (Zucchi, 2000a; Zart et al., 2011).

The species *Anastrepha striata* is considered an endemic pest in the Amazon region, infesting fruit of more than 16 families of host plants (Massaro Júnior et al., 2011). This species has been considered a pest of great relevance within the Myrtaceae family, mainly in the production of guava (*Psidium guajava* L.), and it is constantly found in studies of population fluctuation and index of infestation of this fruit growing (Silva et al., 2007; Silva et al., 2011; Weems Júnior et al., 2013). According to Trassato et al. (2015), *A. striata* was the most found species in guava orchards in the municipality of Boa Vista - RR, presenting population growth in the fruiting period due to the greater availability of fruit in the orchards.

The study of the sexual behavior of *A. striata* is of great relevance and can help in the development of new methods for the management and control of this pest (Almeida et al., 2013). Aluja et al. (1993) observed the differentiated behavior of the species *A. striata*, such as the formation of female-male pairs that interact for prolonged periods, the interaction movements that precede the copula, period on the day of greatest occurrence of oviposition.

The understanding of the biological cycle and the behavior of this insect-pest allows improving and adapting the use of techniques such as the use of sexual attractives in monitoring through the use of traps, besides making possible the use of the sterile male technique, which has the objective of reducing populations of insect-pests, by reducing the reproductive potential of wild females through the release of sterile males to compete with wild males (Walder, 2000; Vera et al., 2013).

Due to the few studies carried out with *A. striata*, there is need of studies that analyze the behavior of this pest species, verifying their sexual behavior in the laboratory for a better development of techniques for the creation, management and control of this tephritid.

Material and Methods

Obtaining the fruit

The Japanese Guava fruit were used to obtain wild *A. striata*. The samples were taken in an orchard with a 3 ha area, with 577 plants, spaced 6 × 6 m, located in the region of Bom Intento (02° 53' 49,7'' N e 60° 39' 35,7'' W), a municipality of Boa Vista, Roraima. The fruit collections were carried out in the months of September and October of 2015.

Obtaining adults of *Anastrepha striata*

The collected fruits were taken to the Plant Protection Laboratory located at the Center of Agrarian Sciences (CCA) of the Federal University of Roraima (UFRR), where they were washed with distilled water and placed to dry on white towel paper.

They were then deposited in plastic containers containing 2 cm of sterilized sand as a substrate. To facilitate the circulation of air into the vessel, the caps were cut (15 cm in diameter) and then white voile tissue was glued (Figure 1).

The sand inside the containers was sieved every day by using a 1 mm mesh sieve, for a total period of 10 days to obtain the pupae.

The pupae were conditioned in transparent acrylic cups (150 mL), covered with white voile tissue, containing 2 cm of sterilized and moist sand to obtain the adults.

Figure 1. Plastic containers containing sand sterilized as a substrate and covered with voile tissue, in which the fruit were deposited

Maintenance conditions

The insects were kept in laboratory in glass cages (30x30x30cm) under controlled conditions of temperature (26 ± 2° C), humidity of 80 ± 5% and lighting regime organized in light/dark cycle of 12/12 hours. The light phase intensity was 750 lux, equivalent to two fluorescent lamps of 32w, which were lit between 7 a.m. and 7 p.m.. The dark phase had intensity of less than 1 lux, using red incandescent bulb of 25 w, being lit from 7 p.m. to 7 a.m..

Study of maturation and sexual activity

Seven virgin couples of *A. striata*, of the same age, were individualized in two-liter polyethylene terephthalate (PET) cages to observe their sexual maturation (Figure 2), containing water and artificial diet (brown sugar and beer yeast, in the ratio of 3: 1) (Braga Sobrinho et al., 2009).

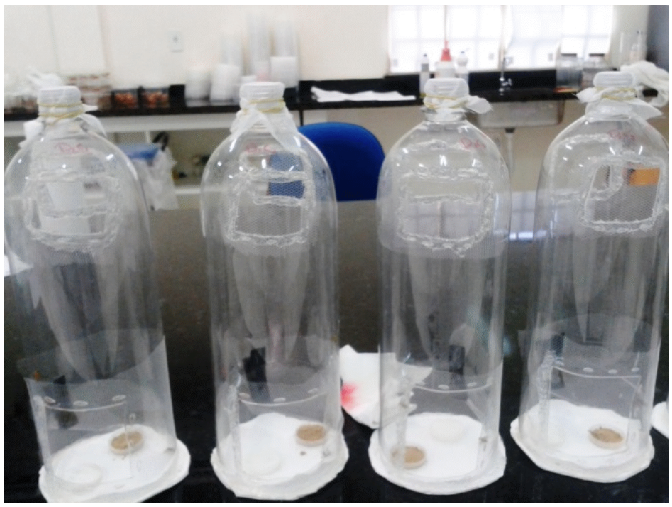

Figure 2. Cages made from 2-liters polyethylene terephthalate (PET) bottles to observe the sexual behavior of *A.striata*

It was considered the beginning of sexual maturation: the distension of the abdominal pouches in males (Figure 3A) and the occurrence of mating in females (Figure 3B) (Facholi-Bendassoli & Uchôa-Fernandes, 2006).

Observations were made daily between 1 p.m. and 7 p.m. during the reproductive period. Observations occurred for 30 minutes at intervals of 15 minutes, and the entire evaluation period was filmed and later studied, aiming to determine the date of reproductive behavior, which characterizes the sexual maturity of individuals (Facholi-Bendassoli & Uchôa-Fernandes, 2006).

After observing that the females were sexually mature, researchers determined the frequency and amplitude (duration) of sexual activity, as well as the behavioral characteristics presented by the individuals for a period of fifteen days.

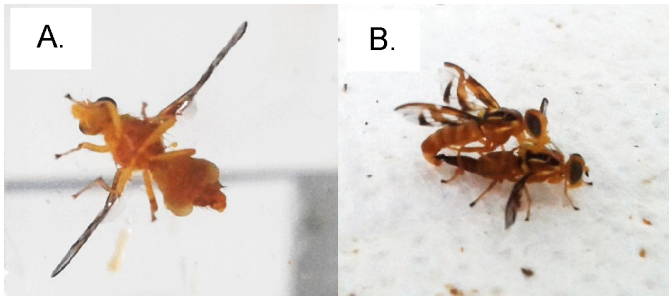

Figure 3. (A) Distension of abdominal pouch in males and (B) mating

Data analysis

Data on the age of sexual maturation of females and males of *A. striata* were submitted to analysis of variance. The information regarding the amplitude (duration) of the sexual activity of call was submitted to analysis of variance (ANOVA) by using the Scott-Knott test ($p < 0.01$ of significance) for comparison between the days of observation, by using the statistical program Sisvar.

The information regarding the period of sexual activity was analyzed by Kruskal Wallis non-parametric test at 1% significance level to compare the variation of sexual activity in each of the observation periods, since the variance was not homogeneous and not all the data from the samples presented a normal distribution.

Results and Discussion

Sexual maturation

Males of *A. striata* reached sexual maturity between 12 and 16 days after emergence, and most individuals became sexually mature at 14 days (Figure 4). During this period, the males first distended the abdominal pouches, located between the 3rd and 5th segments, probably due to the production of hormones, as described by Facholi-Bendassolli & Uchôa-Fernandes (2006).

According to Nation (1989) this aspect was observed in studies with *Anastrepha suspensa*, and there is a relationship between sexual behavior, pheromone release and the development of abdominal pouches in that abdominal region.

Females of *A. striata* reached sexual maturation from 16 to 19 days after emergence, most of them becoming sexually mature at 17 days of age (Figure 4), when they mated for the first time.

It was observed that the mating of *A. striata* males cannot be used as evidence of sexual maturation, since males may be sexually mature and do not mate due to the difference in the age of sexual maturation of females or due to the preference of females for individual morphological characteristics of the male. These differences had been already found by Almeida et al. (2013), when studying the influence of some morphological characteristics in the choice of males of *A. zenildae*. These authors observed that the preference of the females is related to the size of the corporal structures of the males of this species.

There were significant differences between the ages of sexual maturation of males and females of *A. striata* (Anova, $F = 23.43$ and $p \leq 0.001$). The males presented faster development (Figure 4), characterizing the protandry in the sexual maturation of this species. Such behavior was also observed by Facholi-Bendassolli & Uchôa-Fernandes (2006), when studying the sexual behavior of *A. sororcula* in laboratory, in which males matured sexually first (between 7 and 18 days) than females (between 14 and 24 days).

In this work, the mating was considered as determination of the age of sexual maturation of females. Further research must be carried out aiming at determining more characteristics that better evidence the age of sexual maturation, since mating can be delayed due to the partner selection system.

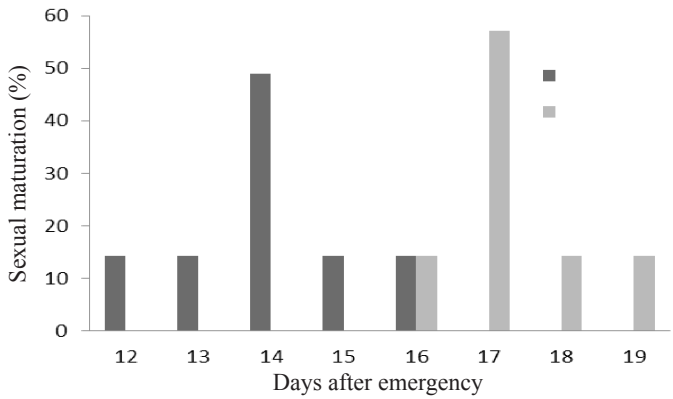

Figure 4. Sexual maturation of *Anastrepha striata* males and females in laboratory (n = 7 couples) (Anova, CV=8.90%, F=23.43 e p≤ 0.01)

Sexual behavior

The sexually active *A. striata* males, positioned on the walls of the cages in a vertical position, signaled to the females by stretching their abdominal pleuritos of the 3rd and 5th segments, in which a small pouch was formed on each side of the abdomen, and they extended a tiny membranous pouch of rectal cuticle that surrounds the anal area (Figure 5A). During this process, named call or courtship, males usually vibrated their wings rapidly, producing audible and repetitive sounds.

Such behavior was also observed by Takata (2010) in several species of the genus *Anastrepha*. However, in *A. suspensa* no evidence of sexual dimorphism was found in the movement and wings pattern (Sivinski & Pereira, 2005). According to Sivinski et al. (1984) the rapid movements of the wings produce an acoustic frequency as sexual signals and can also serve as a disperser of sex pheromones.

A small drop, probably of pheromone, was released from the anal region during the wings vibration. In the period of greatest sexual activity, the males rotated the body 360 degrees repeatedly, while flapping their wings and touching the anal membrane several times on the cage wall, leaving a tiny drop.

The males remained in signaling activity until an attracted female approached. During the courtship, the females approached frontally of the males, extending their wings horizontally with the upper side prostrated forward, moving them up and down (Figure 5B). The male remained immobile until the female touched the non-pungent sucking lip (Figure 5C). The male then jumped over the female's back and mounted (Figure 5D). The males positioned their hind legs close to the female's aculeus so as to raise it so that the aculeus was prolonged and in this way the coupling of the genitals could occur. Such behavior has already been observed by Dickens et al. (1982) in *A. ludens*, by Silva et al. (1985) in *A. obliqua* and by Facholi-Bendassolli & Uchôa-Fernandes (2006) in *A. sororcula*.

In the various attempts of copulation, the females rejected the males, lowering the ovipositor so that the insertion of the male's genital did not occur, besides moving the wings to prevent the males from approaching. When the female accepted the male approach and allowed him to raise her ovipositor to perform the copulation (ventral region of the male's thorax over the female's dorsal region), both remained standing for 10.96 ± 1.64 min (Figure 5D), moving when disturbed or after separation of the genitals at the end of mating.

The separation of the genitalia was initiated by the movement of the hind legs along the ovipositor and by the movement of the female's wings, in order to detach her from the male that held her. In this way, the male moved away from the female and the two, in diametrically opposed position, walked slowly to the decoupling of the genitals. Then they used their hind legs to cleanse the genitals.

The behavioral study is fundamental for the understanding of the isolation of the species. Selivon & Morgante (1997) observed that the species *Anastrepha bistrigata* (Bezzi, 1919) and *A. striata*, which belong to the taxonomic group "striata", present reproductive isolation, male courtship, female acceptance and the entire mating process of *A. striata*, does not occur in *A. bistrigata*, which is one of the factors that prevents the choice and agreement of the female with copulation.

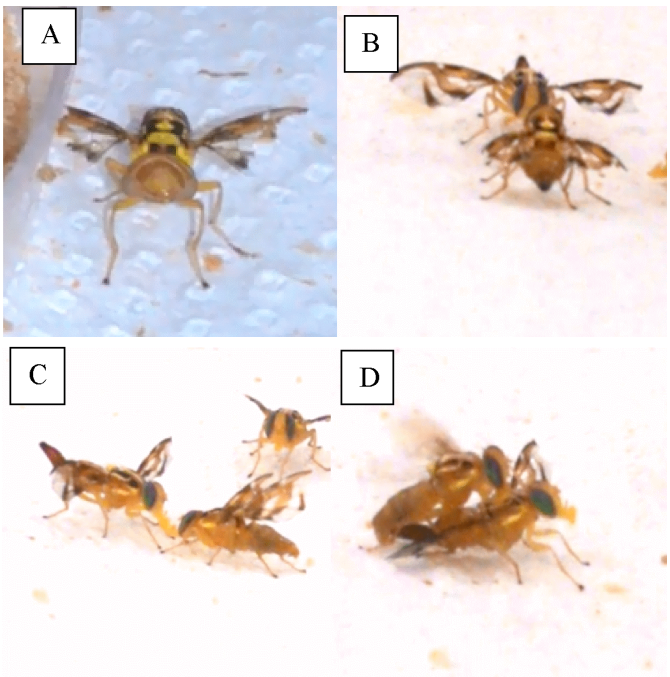

Figure 5. Typical sequence of reproductive behavior of *Anastrepha striata*. (A) The male fly signals to the female with wings vibration, distension of the abdominal pouches and eversion of the anal pouch, (B) the female approximates extending her wings horizontally with the upper side prostrated forward, (C) the male remained immobile until the female touched him with a non-pungent sucking lip, (D) the male flies and rides on the female attempting intercourse, using his hind legs to lift the female's ovipositor for the coupling of the genitalia

Periodicity and duration of sexual activity

The beginning of the call of the males in the laboratory occurred from 1:30 p.m. and reached its maximum at 3:30 p.m., decreasing at 6:30 p.m.. The copulation activity of *A. striata* began at around 1:30 p.m., remaining stable until 4:30 p.m. and reaching the peak between at 5 p.m. and 6 p.m (Figure 6), where the females were more sexually active. Sexual behavior declined sharply at 6:30 p.m..

Such behavior was also observed in *A. obliqua,* which presented copula peak between 5 p.m. and 6 p.m in semi-natural condition (Malavasi, 1984), corroborating with the data obtained in this research. However, for other species

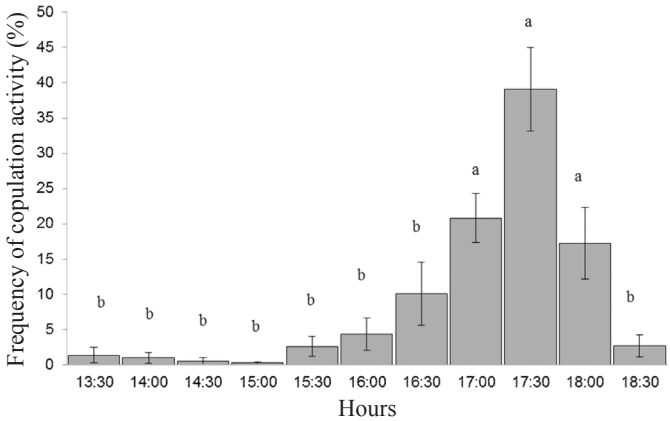

Figure 6. Daily rhythm of the sexual activity of *Anastrepha striata* in laboratory. Mean values ± standard error. The bars followed by the same letter do not differ significantly from each other (Kruskal Wallis, P≤0.05) (N = 7 couples)

of the genus *Anastrepha*, the copulation schedule may vary. For *A. sororcula*, the copulation schedule occurred between 6 p.m. and 7 p.m. in the laboratory (Aluja et al., 1999; Facholi-Bendssolli& Uchôa-Fernandes, 2006), whereas for *A. fraterculus*, it occurred between 7 a.m. and 10 a.m. in semi-natural conditions (Malavasi, 1984). On the other hand, *A. serpentina* was sexually active throughout the day (between 8 a.m. and 6:30 p.m.) both in the field and in the laboratory (Facholi-Bendassolli& Uchôa-Fernandes, 2006).

According to Walder (2000), the determination of the time of greater sexual activity is important information for the use of control management techniques, such as the use of sterile male, making possible the reduction of the populations of these insect-pests.

The mean daily duration of call activity among males of *A. striata* was 3.74 ± 0.037 h (Figure 7). The mean daily activity of the call was significantly influenced by the male's age, and the highest call periods were observed when males were on the 18th and 19th days after emergence (Scott-Knott, p≤0.01) (Figure 7).

According to Facholi-Bendassolli & Uchôa-Fernandes (2006), the calling behavior of *A. sororcula* males can be observed between the 18th and 22nd days after emergence.

The results observed in the sexual behavior of *A. striata* provide important information for the progress of research related to the management of this pest, since by determining the period of sexual activity of males, it is possible to use techniques such as extraction of sex pheromones for use in traps.

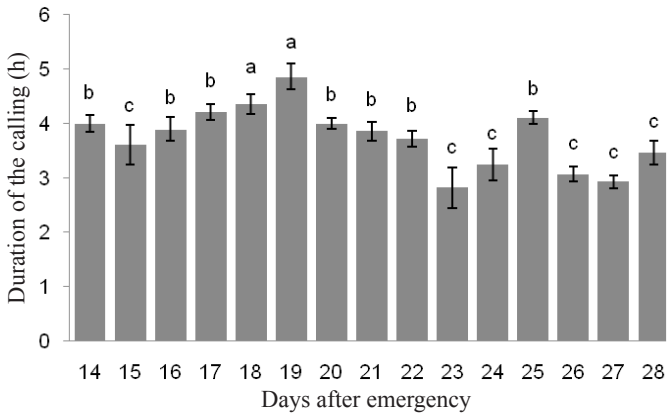

Figure 7. Duration of the calling behavior of the males of *Anastrepha striata* in the laboratory. Mean values ± standard error. The bars followed by the same letter do not differ from each other (Scott-Knott p≤0.01) (n = 7 couples)

Conclusions

The males of *A. striata* reached sexual maturity between 12 and 16 days after emergence, exhibiting signaling behavior to females, characterized by distension of the abdominal bags.

The period of call of males is characterized by rapid movements of their wings, with production of audible signals and diverse movements of courtship.

Females reach sexual maturity between 16 and 19 days after emergence.

Daily sexual activities, in laboratory condition, occur more frequently in the period between 5 p.m. and 6 p.m.

Anastrepha striata presented a marked protandry pattern.

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