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Bioassay

Oviposition behavior of *Anastrepha obliqua* (Maquart, 1835) (Diptera: Tephritidae) and preference between two mango (*Mangifera indica* L.) varieties

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Abstract. *Anastrepha obliqua* (Macquart, 1835) (Diptera: Tephritidae) is a species of fruit fly that occurs in the Neotropical region and is associated with 78 host plants. In Brazil, the species is one of the most economically important pests for fruit growers, especially in mango cultivation in the northeast region. Evaluating the oviposition behavior of polyphagous species such as fruit flies contributes to a better understanding of their fruit choice and infestation patterns and provides a basis for developing pest management techniques. In this study, we evaluated the changes in oviposition behavior of *A. obliqua* at different times of day and fruit fly ages with the highest oviposition activity. In addition, we tested whether *A. obliqua* exhibits any preference for Tommy Atkins and/or Palmer mango varieties. Our results showed higher oviposition activity in the morning and at the ages of 15-16 and 17-18 days. We also observed a preference among females to lay eggs on Tommy mangoes over Palmer ones.

Keywords: Fruit flies, Trypetinae, pest insects, host preference.

The fruit fly *Anastrepha obliqua* (Macquart, 1835) (Diptera: Tephritidae) has a Neotropical origin, with a range extending from Mexico to South America (Fu et al. 2014). This species is associated with 78 hosts (Norrbom 2022) and has a preference for fruits of the family Anacardiaceae, such as *Spondias* spp. and mango (*Mangifera indica* L.) (Weems et al. 2012; Hernández et al. 2019; Oliveira et al. 2022). It is considered the most significant pest of mango crops in South America (Weems et al. 2012; Zucchi et al. 2023). In Brazil, *A. obliqua* is widely distributed and is associated with 70 hosts (Zucchi & Moraes 2023), with its most significant impact observed in the northeast (Haji et al. 2005), where the mango production is concentrated (Gazzola et al. 2020).

Mangoes are originally from India and are currently produced on several continents (Yadav & Singh 2017), with major areas under cultivation in Brazil and Mexico, where exports grew rapidly between 2008 and 2018 (Gazzola et al. 2020). In Brazil, the mango-growing hub of the San Francisco River Valley is located mainly in the northeast region, in the states of Bahia and Pernambuco. San Francisco Valley is responsible for the country's most significant production and export of mangoes, exporting 272,500 tons in 2021 (Abrafrutas 2022). The main cultivar in San Francisco Valley is Tommy Atkins, which accounts for more than 70% of the region's production, followed by the cultivars Haden, Palmer, and Keitt (Pinto et al. 2004).

The distribution of fruit flies — as well as their host selection and the success of larval development — is directly related to host availability and intrinsic characteristics (Joachim-Bravo et al. 2001; Copeland et al. 2006), such as the fruit fly species, fruit species, type of cultivar, maturation stage, volatile compounds, and chemical defenses (Cunningham et al. 2016; López-Ley et al. 2016; Hernández et al. 2019; Guillén et al. 2022; Louzeiro et al. 2022). Therefore, evaluating the oviposition behavior of polyphagous pests like fruit flies contributes to a better understanding of their patterns of fruit choice and infestation and provides the basis for developing pest management techniques (Roitberg 2007).

We aimed to evaluate the oviposition behavior of *A. obliqua*, focusing on age and preferred time of day for oviposition and their preference between two varieties of *M. indica* (Tommy Atkins and Palmer).

The experiments were carried out with specimens of *A. obliqua* (F > 30) from the colony maintained at the Laboratory of Insect Behavioral Ecology, Federal University of Bahia. Adults were kept in plastic cages (15 x 11 x 9 cm) under controlled conditions (temperature = 25 ± 1 °C; humidity = 80 ± 10%; photoperiod = 12h). A diet contained hydrolyzed protein and sugar (1:3) (Silva-Neto et al. 2012) and water were offered *ad libitum*. To maintain the cycle of these insects, mango fruits (*M. indica* var. Tommy Atkins and Palmer) were offered to sexually mature females (± 15 days old) for 48 h. The infested fruits were placed in plastic trays containing vermiculite and periodically monitored until larvae were no longer detected. The puparia were then placed in acrylic pots containing moistened vermiculite in a dark environment until the adults emerged.

The oviposition experiments were carried out in screened cages (49 x 45 x 49 cm). The females were separated into three groups (with 10 females each) according to their age (13-14 days; 15-16 days; 17-18 days). The flies from each group were released in the morning (8 am) into a screened cage containing 10 grapes (*Vitis* sp., Italia variety) as a substrate for oviposition. The fruits were offered to females from 8 am to 12 pm. The grapes were removed and a new set of 10 grapes was offered from 1 to 5 pm. Ten replicates were performed for each age group. The data from this experiment were analyzed using a Generalized Linear Model with a Poisson distribution, considering age (13-14 days; 15-16 days; 17-18 days) and time of day (morning and afternoon) as fixed factors and the number of eggs as a response variable. The significance of the model was checked using the Type III Wald Chi-squares or F-test ($\alpha = 0.05$).

To compare the oviposition preference of *A. obliqua* between

the two commercial mango varieties (Tommy Atkins and Palmer), 10 15-day-old females (sexually mature and mated) were released into a screened cage (49 x 45 x 49 cm) containing one fruit of each mango variety. The fruits of both varieties, obtained in local supermarkets, were used in the green stage of ripeness (peel predominantly green), the most accepted stage for oviposition by females in our preliminary experiments. The experiments took place between the hours of 8 am and 12 pm. Ten replicates were performed, and the data were analyzed using the same model described in the prior experiment, considering mango variety as a fixed factor and the number of eggs as the response variable.

Our results showed oviposition was greater in the morning than the afternoon ($F = 30.03$, $df = 1$, $p < 0.0001$) (Fig. 1A). Furthermore, we observed significant difference in the number of eggs laid by females at different ages ($F = 24.35$, $df = 2$, $p < 0.0001$), with a higher number of eggs observed in the age ranges of 15-16 and 17-18 days (Fig. 1B). No significant interaction was observed between age and period of day ($F = 1.40$, $df = 2$, $p = 0.25$). Finally, females of *A. obliqua* laid more eggs on Tommy Atkins than on Palmer mangoes ($F = 14.92$, $df = 1$, $p = 0.001$) (Fig. 2).

In our experiments, *A. obliqua* showed a preference for oviposition in the morning (8-12 am). According to Flitters (1964); Raghu et al. (2002) and Raghu et al. (2004), there is a positive influence of light intensity/time of day on the number of fruit flies on the host, especially females, which seems to be consistent with the behavior observed in the present study. However, the oviposition patterns differ by species. A laboratory experiment showed that light incidence strongly influences the oviposition activity of the Mexican fruit fly *Anastrepha ludens* (Loew, 1862) (Diptera: Tephritidae) (Filtters 1964). In addition to our results, Guillèn et al. (2022) demonstrated that although *A. obliqua* presents a diurnal oviposition behavior, it prefers to oviposit in shaded fruits.

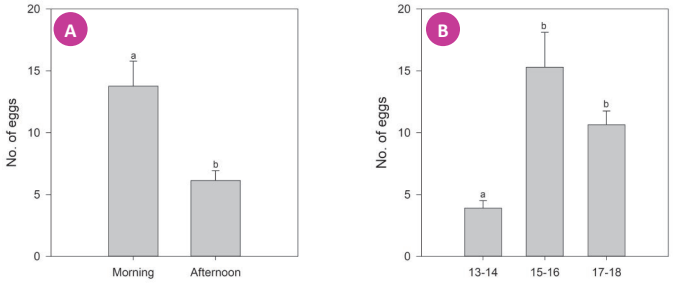

Figure 1. Oviposition activity of *A. obliqua* at different day periods and ages. (A) Mean (±SE) number of eggs laid in the morning (8-12h) vs. afternoon (13-17h). (B) Mean (±SE) of eggs laid by females of three different ages: 13-14, 15-16, and 17-18 days. Data represent the means of 10 replicates from each experiment. GLMs evidenced significant differences between the two-day periods tested ($F = 30.03$, $df = 1$, $p < 0.0001$) as well as between the ages tested ($F = 24.35$, $df = 2$, $p < 0.0001$).

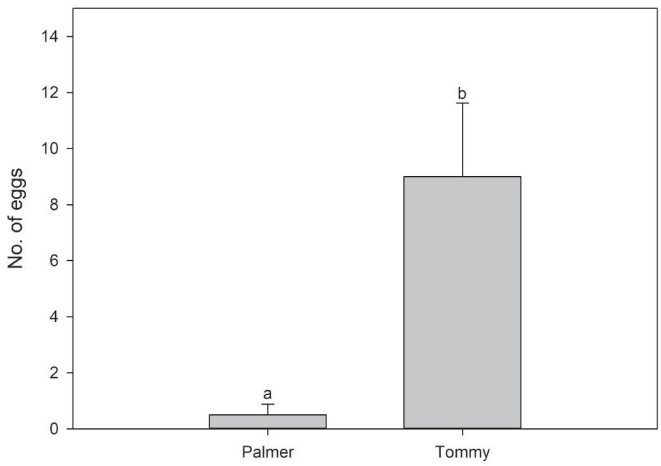

Figure 2. Oviposition preference of *A. obliqua* between two mango varieties (Tommy Atkins and Palmer). Data represent the means (±SD) of 10 replicates. GLM evidenced a higher number of eggs laid on the Tommy Atkins variety in relation to Palmer ($F = 14.92$, $df = 1$, $p = 0.001$).

Among the evaluated ages, the peak of oviposition of *A. obliqua* occurred at ages of 15-16 and 17-18 days. Our data agree with the findings of Joachim-Bravo et al. (2003) and Silva et al. (2020) for *A. obliqua*. Both studies recorded peak oviposition for *A. obliqua* at 18 days. Oviposition peaks at similar ages have also been observed in other tephritid species, such as *Anastrepha fraterculus* (Wiedemann, 1830), *Anastrepha zenildae* Zucchi, 1979, and *Anastrepha sororcula* Zucchi, 1979 (Diptera: Tephritidae) (Joachim-Bravo et al. 2003). For the Mediterranean fruit fly *C. capitata*, the oviposition peak occurs at 15 days (Silva et al. 2020).

When both mango varieties were simultaneously offered to females, we found that *A. obliqua* prefers to lay eggs in the Tommy Atkins than Palmer mangoes. Rossetto et al. (2006), Rossetto et al. (2009) and Aluja et al. (2014) suggested that var. Tommy Atkins is susceptible to *A. fraterculus*, *A. ludens*, *A. obliqua*, and *C. capitata*. Although var. Palmer is a host of *A. obliqua* (Rossetto et al. 2009), this cultivar is considered tolerant to the pest's attacks and it appears to be unfavorable for immature development (Aluja et al. 2014), with low levels of infestation compared to other cultivars (Rossetto et al. 2009). Our data corroborate the reported infestation data, indicating that *A. obliqua* females can recognize and choose suitable hosts for their offspring despite being polyphagous.

These results also suggest that *A. obliqua* has a morning oviposition behavior and a preference for var. Tommy Atkins compared to var. Palmer. The data presented here provide a better understanding of the species' behavior and preference for commercial hosts, providing additional information that can be used to develop and improve rearing and integrated pest management techniques.

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Authors' Contributions

ISJ conceived the project and designed the experiments. BSM and CPO performed the experiments and collected the data. FLG and ASA analyzed the data and wrote the first version of the manuscript. All authors read and contributed to the final version of the manuscript.

Conflict of Interest Statement

The authors declare no conflict of interest.

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