Ovipositional Behaviour of Two Fruit Flies, *Ceratitis capitata* and *Anastrepha fraterculus*, in Seven Fruit Hosts in the Laboratory

Ester Marques de Sousa¹, Léo Rodrigo Ferreira Louzeiro¹, Miguel Francisco de Souza-Filho¹ and Adalton Raga¹*

¹Instituto Biológico, Secretaria da Agricultura e Abastecimento do Estado de São Paulo. Alameda dos Videiros 1097, 13101-680, Campinas, SP, Brazil.

Authors’ contributions

This work was performed in collaboration among all authors. Author AR designed the study and wrote the first draft of the manuscript. Authors EMS and LRFL contributed to the maintenance of fruit-fly colonies and the development of the experiments. Author MFSF help to evaluate the fly oviposition and take pictures. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2020/v35i1130303

Editor(s):
(1) Dr. Bechan Sharma, University of Allahabad, India.

Reviewer(s):
(1) Gaurang Chhangani, Maharana Pratap University of Agriculture and Technology (MPUAT), India.
(2) Mohammed Ezziyyani, Université Abdelmalek Essaâdi, Morocco.

Complete Peer review History: http://www.sdiarticle4.com/review-history/62598

Original Research Article

Received 26 August 2020
Accepted 02 November 2020
Published 23 November 2020

ABSTRACT

*Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann) are the most commonly found fruit fly species in fruit crops in Brazil. Both polyphagous species show similar host ranges, but specific knowledge regarding the ovipositional preference of either species is scarce. The present study aims to evaluate, in the laboratory, ovipositional behaviours in seven fruit host submitted to infestation by *A. fraterculus* and *C. capitata*. Except for *C. capitata* in Tahiti acid lime, the number of punctures containing eggs exceeded the number of punctures without eggs. The highest values for eggs per puncture were obtained in Fuyu persimmon, for which averages of 4.06 and 50.09 eggs per puncture were deposited by *A. fraterculus* and *C. capitata*, respectively. For *A. fraterculus*, the infestation ranking, based on the number of eggs per puncture from high to low, was Fuyu persimmon > papaya > Tahiti acid lime > carambola > coffee > guava > sweet orange. For *C. capitata*, the infestation ranking was Fuyu persimmon > carambola > papaya > guava > sweet orange > coffee > Tahiti acid lime. All punctures made to carambola and papaya contained eggs.

*Corresponding author: E-mail: adalton.raga@sp.gov.br, adalton.raga@gmail.com;
Sweet orange exhibited the maximum number of punctures of *A. fraterculus* without egg deposition. The first day of medfly oviposition in Fuyu persimmon resulted in approximately three-fold more eggs per puncture than the second, third, and fourth days.

Keywords: Tephritidae; egg masses; Tahiti lime, carambola; Fuyu persimmon.

1. INTRODUCTION

Female insects choose where to lay eggs on any substrate [1] because this process is the most important event in the lives of female insects [2]. Therefore, host preference is defined as the hierarchical ordering of host plants by ovipositing females [3]. Polyphagous Tephritidae species have received the most attention in research examining host selection behaviours, but the data are rarely robust enough to define the host status [4]. A number of behavioural traits are influenced by egg load, including the persistence with which females forage for oviposition sites, the probability that a host is accepted, and even the size of a female’s clutch [5], once the skin puncture resistance can serve as an important deterrent to oviposition [6]. We would expect plasticity in ovipositional behaviours associated with the environmental context [1] and a variety of factors, including fly density, age, nutrition, egg load, fruit size and ripeness, the existence of previous punctures, and even weather are expected to guide a fly’s egg laying decisions [7]. Moreover, flies also are more likely to oviposit in a host fruit in the presence of a conspecific ovipositing fruit fly than in the absence of other flies [8].

In general, females of the frugivorous Tephritidae deposit oviposition-deterring pheromones on the fruit surface immediately after oviposition, to reduce the probability of intraspecific competition between maggots for the food supply [9]. The choice among available hosts and the oviposition rate is also likely to be influenced by the supposed host quality for larval development [10,11]. The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), marks its hosts (pheromone) and yet prone to multiple oviposition in those fruit hosts, despite the presence of naturally deposited host-marking pheromones [12].

*Anastrepha fraterculus* (Wiedemann) and the exotic *C. capitata* are the main primary fruit flies (Tephritidae) in Brazil [13] and have been recorded to oviposit in 116 and 94 hosts, respectively [14, 15]. However, data regarding fruit fly oviposition are rare in the literature, especially for *A. fraterculus*. This study was conducted to increase our knowledge of the ovipositional behaviour of both fruit flies, which will assist the management of pest populations in different hosts and provide basic information for pest risk analysis and plant health services.

The objective of this study was to evaluate the ovipositional behaviour in seven fruit hosts submitted to infestation by *A. fraterculus* and *C. capitata*: carambola (*Averrhoa carambola* L.), coffee (*Coffea arabica* L.), guava (*Psidium guajava* L.), papaya (*Carica papaya* L.), persimmon (*Diospyros kaki* L.), sweet orange (*Citrus sinensis* L. Osbeck), and Tahiti lime (*Citrus latifolia* Tanaka), under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Fruit Fly Colonies and the Location of the Experiment

Adults of *A. fraterculus* and *C. capitata* were obtained from a culture maintained since 1993 by the Economic Entomology Laboratory, Instituto Biológico, in Campinas, SP, Brazil [16]. After emergence, flies were provided with water and a mixture (w/w) of sugar (49.1%), brewer’s yeast (24.5%), yeast extract (12.2%), wheat germ (12.2%), and Sustagen® (2.0%).

2.2 Infestation Bioassays

2.2.1 Test 1

All tested fruits in the experiments were either ripe or full-ripe (Table 1), based on external visual fruit colour, and were washed thoroughly under running tap water before testing. Due to differences in fruit sizes among the fruit species, we establish the following numbers of fruits per infestation cage: carambola (10), coffee cv. ‘Mundo Novo’ (75), guava cv. ‘Tailandesa’ (8), papaya (3), persimmon cv. ‘Fuyu’ (4), sweet orange cv. ‘Pera’ (10), and acid lime cv. ‘Tahiti’ (20). An additional sample of the fruits without infestation was kept in the laboratory to check the possibility of pre-harvest oviposition.
Table 1. The number of eggs per puncture (n = 100) for two fruit fly species (Tephritidae) in seven fruit hosts, under laboratory conditions

<table>
<thead>
<tr>
<th>Fruit host</th>
<th>Ripe (R) or Full ripe (FR)</th>
<th>Eggs per puncture (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carambola</td>
<td>R</td>
<td>1.42 ± 0.07cB</td>
</tr>
<tr>
<td>Guava</td>
<td>R</td>
<td>1.13 ± 0.05cB</td>
</tr>
<tr>
<td>Papaya</td>
<td>R</td>
<td>2.69 ± 0.13bB</td>
</tr>
<tr>
<td>Coffee</td>
<td>FR</td>
<td>1.16 ± 0.05cB</td>
</tr>
<tr>
<td>Sweet orange</td>
<td>R</td>
<td>0.75 ± 0.08dB</td>
</tr>
<tr>
<td>Fuyu persimmon</td>
<td>FR</td>
<td>4.06 ± 0.29A</td>
</tr>
<tr>
<td>Tahiti acid lime</td>
<td>R</td>
<td>1.47 ± 0.14cA</td>
</tr>
</tbody>
</table>

Original means within a column followed by the same letter and means within a row followed by the same upper case letter do not significantly differ by Tukey’s test (P ≤ 0.05).

Each fruit species was randomly placed on the infestation cage floor (50 cm length; 40 cm height; 40 cm width) and separately exposed to 200 mature females over 24 hours. The ages of the females ranged from 12–15 days-old for A. fraterculus and 8–10 days-old for C. capitata. Water and the standard diet described above for adult flies were provided in each cage. The average humidity, temperature, and photophase in the laboratory were maintained at 56%–61%, 19.2–23.8°C, and 14 h, respectively.

2.2.2 Test 2

In a second experiment, three full-ripe persimmons were daily exposed to medfly females (8-day-old) in an infestation cage for four days to measure whether egg-laying decreased over time. The fruit was exposed to 10 females per fruit in the same cage. The test was conducted using the conditions described for Test 1, and the number of punctures (n=50) was evaluated daily.

2.3 Evaluation and Statistical Analysis

During Test 1, 100 punctures for each infested fruit species and during Test 2, 50 punctures by C. capitata in ‘Fuyu’ persimmon were evaluated. Punctures were examined under a stereoscopic trinocular microscope (Carl Zeiss Stemi 2000-C), by counting the number of eggs per puncture. Images of the punctures and eggs were obtained using a digital camera (Canon Power Shot A650 IS) attached to the microscope.

The results were analysed using a two-way analysis of variance (ANOVA) to compare the number of eggs per puncture by both species of fruit flies in different fruit species (Test 1). A one-way ANOVA was used to analyse the puncture data in persimmon (Test 2). We transformed the data using the square root (x + 1), and ANOVAs were performed using Sisvar, version 5.7 [17], with significance set to P < 0.05. Comparison between treatments was made using Tukey’s test.

3. RESULTS

3.1 Test 1

The highest numbers of eggs per puncture were obtained in Fuyu persimmon, with averages of 4.06 and 50.09 eggs per puncture by A. fraterculus and C. capitata, respectively (Table 1). For A. fraterculus, sweet orange showed the minimum number of eggs per puncture (0.75); whereas, for medfly, Tahiti acid lime showed the minimum number of eggs per puncture (0.10).

Carambola, guava, coffee, and Tahiti acid lime exhibited similar values for mean eggs per puncture by A. fraterculus and C. capitata, respectively (Table 1). For A. fraterculus, sweet orange showed the minimum number of eggs per puncture (0.75); whereas, for medfly, Tahiti acid lime showed the minimum number of eggs per puncture (0.10).

Carambola, guava, coffee, and Tahiti acid lime exhibited similar values for mean eggs per puncture by A. fraterculus and C. capitata, respectively (Table 1). For A. fraterculus, sweet orange showed the minimum number of eggs per puncture (0.75); whereas, for medfly, Tahiti acid lime showed the minimum number of eggs per puncture (0.10).

Experiments were performed using Sisvar, version 5.7 [17], with significance set to P < 0.05. Comparison between treatments was made using Tukey’s test.

Carambola, guava, coffee, and Tahiti acid lime exhibited similar values for mean eggs per puncture by A. fraterculus and C. capitata, respectively (Table 1). For A. fraterculus, sweet orange showed the minimum number of eggs per puncture (0.75); whereas, for medfly, Tahiti acid lime showed the minimum number of eggs per puncture (0.10).

Experiments were performed using Sisvar, version 5.7 [17], with significance set to P < 0.05. Comparison between treatments was made using Tukey’s test.

3.1 Test 1

The highest numbers of eggs per puncture were obtained in Fuyu persimmon, with averages of 4.06 and 50.09 eggs per puncture by A. fraterculus and C. capitata, respectively (Table 1). For A. fraterculus, sweet orange showed the minimum number of eggs per puncture (0.75); whereas, for medfly, Tahiti acid lime showed the minimum number of eggs per puncture (0.10).

Experiments were performed using Sisvar, version 5.7 [17], with significance set to P < 0.05. Comparison between treatments was made using Tukey’s test.

Carambola, guava, coffee, and Tahiti acid lime exhibited similar values for mean eggs per puncture by A. fraterculus and C. capitata, respectively (Table 1). For A. fraterculus, sweet orange showed the minimum number of eggs per puncture (0.75); whereas, for medfly, Tahiti acid lime showed the minimum number of eggs per puncture (0.10).

Experiments were performed using Sisvar, version 5.7 [17], with significance set to P < 0.05. Comparison between treatments was made using Tukey’s test.

Carambola, guava, coffee, and Tahiti acid lime exhibited similar values for mean eggs per puncture by A. fraterculus and C. capitata, respectively (Table 1). For A. fraterculus, sweet orange showed the minimum number of eggs per puncture (0.75); whereas, for medfly, Tahiti acid lime showed the minimum number of eggs per puncture (0.10).

Experiments were performed using Sisvar, version 5.7 [17], with significance set to P < 0.05. Comparison between treatments was made using Tukey’s test.

Carambola, guava, coffee, and Tahiti acid lime exhibited similar values for mean eggs per puncture by A. fraterculus and C. capitata, respectively (Table 1). For A. fraterculus, sweet orange showed the minimum number of eggs per puncture (0.75); whereas, for medfly, Tahiti acid lime showed the minimum number of eggs per puncture (0.10).
Fig. 1. Percentage of punctures without eggs by *Anastrepha fraterculus* (AF) and *Ceratitis capitata* (CC) in seven host fruits, under laboratory conditions.

Fig. 2. Percentage distribution of classes for the number of *Anastrepha fraterculus* and *Ceratitis capitata* eggs per puncture in different fruit hosts, under laboratory conditions.
All punctures observed in carambola and papaya contained eggs. Sweet orange exhibited high rate of *A. fraterculus* punctures without oviposition (Fig. 1). The majority of punctures made by *A. fraterculus* in all the fruit hosts showed between 1 and 3 eggs. Most of the egg masses laid by *C. capitata* in carambola, Fuyu persimmon, and papaya exhibited between 11 and 50 eggs (Fig. 2). Fuyu persimmon contained the largest egg masses in a single puncture for both species: 21 eggs for *A. fraterculus* and 161 eggs for *C. capitata*. The majority of punctures made in coffee berries presented up to 3 *A. fraterculus* eggs or 10 *C. capitata* eggs. The punctures and ovipositions on fruit hosts provided by both fruit flies are illustrated in the Fig. 3.

### 3.2 Test 2

Medflies laid eggs over a four-day period and in all punctures made in Fuyu persimmons exhibited at least two eggs. Significant difference in the numbers of eggs per puncture was observed for the first day of oviposition (mean 27.86 eggs) compared to that for remaining days, which were at similar levels (Fig. 4).

A maximum of 84, 33, 19 and 22 eggs of *C. capitata* by puncture were obtained on the first, second, third and fourth day of exposure. The second, third and fourth days of oviposition showed average reductions of 63.4%, 59.6%, and 66.1% in eggs per puncture, respectively compared to that for day 1.

---

![Fig. 3. Oviposition (A, B) and eggs (C, D) of *A. fraterculus* in Tahiti acid lime; puncture (E) and egg masses (F) of *C. capitata* in sweet orange; *A. fraterculus* egg in sweet orange (G); puncture in coffee cherry (H); *C. capitata* egg masses in Fuyu persimmon (I); *A. fraterculus* oviposition in papaya (J); *C. capitata* oviposition in carambola (K); *C. capitata* egg masses in guava (L)](image-url)
4. DISCUSSION

Except for medfly oviposition in Tahiti acid lime, the number of ovipositing events exceeded the number of punctures without eggs for the two tested fly species. A similar result was reported by McDonald & McInnis [18] for *C. capitata* in peach, apple, plum, and grape.

Depending on the larval host rearing, *C. capitata* can lay up to 690 eggs in total [19,20,21] and *A. fraterculus* can lay up to 460 eggs throughout their lifespans [22].

Females of *A. fraterculus* and *C. capitata* differ substantially in clutch sizes. From all tested fruit hosts, 58% of oviposition events for *A. fraterculus* resulted in only one egg; whereas, this rate was 4.6% in *C. capitata*. Eighty-six percent of the punctures made by *Bactrocera latifrons* (Hendel) on pepper, *Capsicum annum* L. contained one egg [23]. These results suggested that the ovipositional behaviours of tephritid flies are variable. Therefore, understanding the various ovipositional aspects associated with the behaviours of each fly species is necessary to understand the population dynamics and management of each individual species.

Similar differences in clutch sizes were observed between *Anastrepha obliqua* (Macquart), which generally oviposits one egg, and *Anastrepha ludens* (Loew), which typically lays up to 40 eggs [24]. *Ceratitis capitata* females lay one to ten eggs per clutch [25]. In contrast, according to our results, multiple oviposition events at the same puncture appeared to be unusual behaviour for *A. fraterculus*.

Although multiple oviposition events are not easily observed characteristics when examining the peels of our tested fruits, medflies may oviposit into a pre-existing puncture because this structure represents a positive stimulus for *C. capitata* egg-laying [12]. Sweet orange presented similar distributions *C. capitata* eggs among the classes of egg masses (Fig. 2). The reasons for high variability in the number of eggs per puncture may be due to the use of the same puncture by multiple females. This behaviour represents a substantial increase in phytosanitary risk for transportation and the commercial sale of medfly fruit hosts, even under lower infestation rates.

The egg loads of *A. ludens* and *A. obliqua* females that were housed with other females
were significantly greater than the egg loads of isolated females [5]. *Anastrepha ludens* females not only produce eggs continuously to be able to quickly respond to egg-laying opportunities, but the eggs they produce are largely fertile [26].

Depending on the fruit host, the social facilitation of egg-laying behaviours among fruit flies [8,27] may stimulate multiple ovipositing events by multiple *C. capitata* females in the same puncture, as noted for *C. capitata* in sweet granadilla *Passiflora ligularis* (Juss.) [28]. Even social facilitation behaviours are not a ubiquitous phenomenon among all fruit flies [7]. In our case, social facilitation behaviour likely stimulated the ovipositional behaviours of *A. fraterculus* in Fuyu persimmon, papaya, carambola, and Tahiti acid lime.

Although the system approach technique, which is used in areas that grow and export papayas, is based on harvesting fruits during maturation stages 1 and 2 [29,30], females of *A. fraterculus* and *C. capitata* deposited higher numbers of eggs in the soft peels of ripe fruits, under laboratory conditions. Diaz et al. [31] obtained the largest numbers of *A. fraterculus* and *C. capitata* eggs in the fruits of mango and papaya, respectively. The authors attributed this behaviour to “pre-imaginal conditioning” because the insects used in the experiment were reared in the mentioned hosts. In the present study, similar behaviours may partially explain the rate of oviposition by *A. fraterculus* in papaya (2.69 eggs/puncture).

The oviposition of *A. fraterculus* and *C. capitata* in citrus fruits occurred in the oil cells of flavedo [32]. Splitter et al. [33] observed a secretion on the surface of lemon, *Citrus limon* L., cv. Eureka and Lisbon, after medfly oviposition, which sealed the puncture and closed the egg cavity. We detected similar symptoms in Tahiti acid lime but not in sweet orange, which shows that nearly all punctures remained open. A group of *A. fraterculus* females stimulated each other to increase ovipositing behaviours, likely influenced by the aromas of peel volatiles produced by Tahiti acid lime [34], which were emitted by earlier punctures. Socially facilitated behaviour was not detected in medflies on Tahiti acid lime, and even though the females landed on the fruit surface and created punctures, they avoided oviposition, exhibiting only five egg masses with a maximum of 3 eggs per clutch.

Many species of fruit may be infested under the pressure of hundreds of ovipositing fruit fly females in a confined setting; however, under field conditions fewer than 5 flies land on any single fruit [35] and either *A. fraterculus* and *C. capitata* detect conspecific and heterospecific infested fruits [36].

A significant influence of host plants was observed on the different developmental parameters of *Zeugodacus cucurbitae* (Coquillett) [37]. Our results suggested that the fruit host may change ovipositional behaviours, either increasing or decreasing the sizes of the egg masses. *Urophora cardui* (L.) females (Tephritidae) are able to measure bud quality and adapt clutch sizes accordingly [38]. Carambola was the second-most preferred fruit host in terms of egg masses when exposed to *C. capitata* (Table 1) and is commonly infested by medfly in the state of São Paulo [39].

Fuyu persimmon, a non-astringent persimmon, presented the highest levels of oviposition for both fruit fly species. Total and soluble tannins were shown to decrease throughout development in Fuyu persimmon [40], which is favourable for the larval development of *A. fraterculus* and *C. capitata* [41]. In contrast, coffee berries showed the lowest mean number of eggs for *A. fraterculus* per puncture, and very small egg masses of *C. capitata* eggs were observed. The ovipositional behaviours of both species in coffee are likely affected by the thin layers of skin and the pulp of the grain, which provide contact between the aculeus and the coffee seed (endocarp) during the ovipositional process. The mesocarp of coffee berries does not facilitate the larger egg masses or provide adequate shelter for many Tephritidae maggots. *Anastrepha ludens* females also adjusted clutch number and size according to host size [42].

Novoseltsev et al. [43] concluded that female fecundity during the maturity stage in medfly follows a constant rate of egg laying. This behaviour was not observed during Test 2, where we detected a strong reduction in the size of the egg masses after the first day of medfly oviposition in Fuyu persimmon. Harris et al. [44] determined the first peak of medfly oviposition to occur at approximately 8 days old, followed by a substantial decline at 10 days old. At the beginning of the experiment in Fuyu persimmon, medfly females were 8 days old. However, we observed an average of one-third the number of eggs oviposited by *C. capitata* per puncture on the second, third, and fourth days compared to that on first day in Fuyu persimmon.
5. CONCLUSION

The results of this study showed that the polyphagous fruit flies *A. fraterculus* and *C. capitata* presented differences in terms of the sizes of the egg masses per oviposition event in the same fruit hosts. Fuyu persimmon and papaya showed the highest mean number of eggs per puncture for both fruit flies, in addition to carambolas for medfly. Guava, coffee berries, and Tahiti acid lime for *A. fraterculus* and, guava and sweet orange for *C. capitata* were considered to be intermediate hosts. Many egg masses observed for *C. capitata* showed high quantities of eggs, which we considered to contain more eggs than are typically produced in a single clutch. Tahiti acid lime demonstrated almost no medfly oviposition. The first day of medfly oviposition in Fuyu persimmon resulted in approximately three times more eggs per puncture than the second, third, and fourth days.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGMENTS

This work was performed with the partial support of the Coordenação de Pessoal de Nível Superior – Brazil (Capes) [Financing code 001].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


34. Ioannou CS, Papadopoulos NT, Kouloussis NA, Tananaki CI, Katsoyannos...
102


© 2020 Sousa et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62598