Sexual and Oviposition Behavior of *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in Cashew Apple (*Anacardium occidentale* L.) †

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Abstract: The host selection behavior is essential to studies of plant-insect interaction, considered as a critical step to populations maintenance since it directly influences the offspring development. This work describes the sexual and oviposition behavior of the invasive species *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in cashew apple (*Anacardium occidentale* L.). The results showed that from 5.645 behavioral patterns registered, for males and females in the tests with papaya, mango, cashew apple, sprayed papaya extract and sprayed mango extract, 3.719 were activities displayed by the males and 1.935 displayed by the females. To the females, the walking activity on the cashew apple differed between the morning and afternoon shift (4.3 + 2.58 and 1.5 + 1.22). The oviposition in mango fruits (11.16) differed from all the other treatments, except from papaya (6.38). However, the quantity of obtained adults was higher in papaya fruits (97) than in mango fruits (49), reducing on the papaya (48) and mango (24) treatments exposed to the cashew apple extract. There are differences in the total number of obtained adults by treatment, showing that the cashew extract reduces the total in papaya and mango treatments.

Keywords: Fruit flies; host selection; semiarid fruits; anacardium occidentale

1. Introduction

Mating and host selection behavior of males and females are important for the investigations related to plant-insect interaction, since they are directly influenced by the presence of plants in the area by chemical and physical signs [1–3].

The selection of host fruit for *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) is displayed by the females, being a critical stage to the population maintenance by directly influencing in the brood development [4]. In general, several host is evaluated to the *C. capitata* females before the oviposition [5]. The oviposition behavior can be divided into four steps: (1) the arrival in the fruit—stage when the insect seek for the most suitable fruit to lay the eggs, relying on visual stimuli, such as color, format and size; (2) Search—chemical and physical analysis of the fruit; (3) Puncture—stage when the insect introduce the ovipositor in the fruit, not necessarily laying the eggs; (4) Dragging—after the oviposition, the female releases a signaling pheromone in order to any other female perceive the fruit is already colonized [6]. The host selection happens from the evaluation of different aspects, inasmuch as some fruit and plant characteristics can influence in the host acceptance...
or rejection, as: maturation stage of the fruit, phenological stage and volatile production of the plant [7]. Size, color, fruit form and the place where they are in the plant also act as visual clues to localize and evaluate the fruit, supporting the choice of the more suitable ones for the oviposition [8].

The reports of *C. capitata* infesting cashew apple fruits in field conditions are rare, especially when it is focused in semiarid regions. The presence of allelopathic components, negatively acting in the adults and larvae, can influence in the low collection of *C. capitata* colonized fruits in the field. Thus, it is necessary to invest in studies about the acceptance of such pseudofruit to the oviposition of *C. capitata*, based on a hypothesis that there is a negative influence coming from the fruit which leads the female to other hosts. In this way, we aimed to verify the sexual and oviposition behavior of *C. capitata* in cashew apple fruits, and the interference of such fruits in the behavior of males.

2. Experiments

*Experiment characterization.* It was carried out tests with fruit fly couples (*Ceratitis capitata*), Vienna 8 strain, between 10 and 12 days (stage when the adults are sexual mature) in order to evaluate their behavior. For that, such visual notes were collected over 12 h in tests. It was used mature fruits from three species: cashew apple (*Anacardium occidentale* L.); mango (*Mangifera indica*); and papaya (*Carica papaya* L.).

The fruit flies were obtained from the previous rearing ([9], methodology) in the Applied Entomology Laboratory from Federal University of Semiarid (UFERSA), Brazil, where were also carried out all the experiments.

*Tests.* It was made tests with cashew apple fruits, papaya and mango without any type of treatment and tests with papaya and mango sprayed with cashew apple fruit extract. The essays were conducted in plastic cages (60 cm in all dimensions). For the treatment essays, before the introduction of the fruits in the cages, they were sprayed with cashew apple juice, previously squeezed and stored in 500 mL plastic containers. Then, the papaya and mango fruits received 2 mL of such extract, in a way where all the fruit surface were covered. From this point on, the fruits were only introduced in the cage after the extract completely dried.

Inside of each cage, it was offered artificial food, water and five fruits. It was also released three couples of *C. capitata* per fruit, summing 15 couples in each cage. They were separated 24 h before the beginning of the experiment. After the acclimatization of the couples in the cages, it was collected behavior data every 30 min, during 12 h, totaling 24 observations per repetition. Each observation lasted 3 min. The first observations were at 06:30 a.m., finishing at 18:00. The essays were conducted in controlled conditions (25°±2°, 60% RH and 12 h photophase).

All the males and females activities were counted and registered. It was observed the main activities: feeding, water consumption, attraction (to the males), courtship, mating, walking on the fruit and oviposition (to the females). It was considered as feeding and water consumption activity those in which the insects repeatedly presented the proboscides touching the food and water container. To the attraction, it was checked if the males were releasing drop of pheromone; and, as courtship, the moment when males and females were interacting, when there is the partner choice to, eventually, mate. The flies which walked on the cage surface without any interaction with other individuals or were simply inactive were registered as rest.

After the last observation, and in order to allow the larval development, the fruits were removed from the cages, stored in plastic containers filled with a thin layer of vermiculite, simulating the soil condition. After 12 days, the vermiculite was removed and it was searched for late pupae and larvae in the fruits. Then, adults were counted after the emergence.

*Data analysis.* Based on the observations, the data were descriptively analyzed. The individual behavior patterns were evaluated for each treatment, dividing them in shifts. The morning shift included the activities observed in the first period (06:30–12:00), while
the afternoon shift was considered the rest of the time (13:30–18:00). Initially, it was observed the confidence interval of the mean per shift, following the P value. After all, it was ran the T test for mean comparison, discriminating which activities/treatments had difference between morning and afternoon shifts.

To the evaluation of mean comparison for the behavioral patterns between the treatments, it was initially used Shapiro-Wilk test, which checks if all means were equal. Confirming the data normality, it was applied the F test (ANOVA), running the global mean comparison. Then, it was applied the Tukey test to the multiple comparison. To the results that did not present data normality, it was made Kruskal-Wallis, thus, comparing if the distribution functions were the same for all the treatments tested. In these cases, it was applied Dunn test with Bonferroni correction to the multiple comparisons. In the comparison of adults obtained per each treatment, it was applied Qui-square, evaluating if the data frequency were equal. In case of hypothesis rejection, it was ran multiple comparisons in column pairs. For all analysis, it was adopted 95% of reliability level, using [10] to develop the analysis, while the graphs were constructed in Excel.

3. Results

It was recorded 5654 activities in total for males and females in papaya, mango, cashew apple fruits, papaya and mango sprayed with cashew extract. From this number, 719 were displayed by the males and 1935 by females. The treatment which presented the higher quantity of activities was the mango one (1388), with 868 recordings for males and 520 for females, followed by the cashew apple treatment (1297 — males: 836; females: 461), papaya (1206 — males: 741; females: 465), sprayed mango (899 — males: 647; females: 252) and sprayed papaya (864 — males: 627; females: 237). For all the treatments, there was a reduction of activities during the first hours of the afternoon shift, presenting an increase after 15h00. Such variation was higher for the males when compared to the females.

To the males, the most frequently behavior was attraction, showing more than 50% from all daily activities displayed by this group. This behavior was generally observed in the upper part of the cage. On the other hand, for the females, the most frequent activity was the courtship (30%). In this case, the only exception was for the papaya, which had the highest number of recordings of walking on the fruit (30.11%).

To the females, the main activity was the courtship. After such behavior, walking on the fruits in the papaya and mango in natura treatments, as well as for the sprayed papaya, was also high. Followed by these activities, there was feeding for cashew apple and sprayed mango treatments.

It was evaluated if the behavioral patterns for each treatment would be different between the shifts (95% reliability level). It was observed that for cashew apple fruit treatment, for females, there was a mean difference between the morning and afternoon shifts, respectively, in the water consumption (7.3 ± 1.6 and 4.0 ± 2.53), courtship (22.0 ± 6.6 and 7.3 ± 2.34) and walking on the fruit (4.3 ± 2.58 and 1.5 ± 1.22). To the males of such treatment, there was difference between the shifts to attraction (57.7 ± 15.3 and 18.2 ± 4.92) and courtship (26.8 ± 8.16 and 9.7 ± 3.14). In all cases, the higher values of recorded activities were for the morning shift (Table 1).

### Table 1. Comparative analysis of behavioral patterns (mean±SD) between shifts (morning and afternoon) for females and males of *Ceratitis capitata* displayed in 12 h light with different oviposition means, by no-choice tests in 25 ± 2 °C temperature and 60% RU environment.

| Behavior Patterns | Cashew Morning | Afternoon | Papaya Morning | Afternoon | Sprayed Papaya Morning | Afternoon | Mango Morning | Afternoon | Sprayed Mango Morning | Afternoon |
|-------------------|----------------|-----------|----------------|-----------|------------------------|-----------|---------------|-----------|------------------------|-----------|
| Females Feeding   | 10.8 ± 3.67    | 6 ± 4.2   | 6.8 ± 3.48     | 7.3 ± 7.42| 3.8 ± 2.27             | 2.7 ± 2.34| 6.5 ± 2.43    | 7 ± 3.58  | 4.7 ± 1.60             | 3.2 ± 1.60|
| Water consumption | 7.3 ± 1.63     | 4 ± 2.53  | 3.8 ± 2.04     | 2.5 ± 1.38| 1.7 ± 1.63             | 0.5 ± 0.84| 3.7 ± 2.34    | 2.8 ± 2.23| 4.2 ± 3.25             | 1.5 ± 1.22|
| Courtship         | 22 ± 6.60      | 7.3 ± 2.34| 11 ± 1.10      | 6.5 ± 2.35| 11 ± 5.25              | 3.8 ± 3.19| 24.7 ± 3.72   | 4.5 ± 1.05| 10.2 ± 4.40            | 6.2 ± 1.33|
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In the papaya fruit treatment, there was a difference for females only in the courtship patterns, with means for morning and afternoon shift of, respectively, 11 ± 1.1 and 6.5 ± 2.35; while the difference between shifts for the males happened in the attraction (55 ± 6.0 and 27.8 ± 5.5) and courtship (11.7 ± 1.97 and 6.7 ± 2.66). On the other hand, for the sprayed papaya fruit, the behavior patterns which did not show an equivalent form for the females were courtship (11 ± 5.25 and 3.8 ± 3.19) and mating (4.8 ± 1.6 and 2.3 ± 2.07), with mean values for the morning and afternoon shifts, respectively. To the males, besides the mating, there were three patterns which showed difference between the shifts: feeding (1.7 ± 1.25 and 0.2 ± 0.41), attraction (48.5 ± 11.2 and 21.5 ± 5.17), and courtship (13 ± 5.14 and 4.5 ± 3.62).

It was also compared the behavior patterns among the treatments. To the females, the feeding pattern was higher in the cashew apple (16.83 ± 5.74); there was difference between this treatment and the patterns found in sprayed papaya and mango (6.50 ± 4.54 and 7.83 ± 1.60, respectively), with no difference among the other interactions. To the water consumption pattern, it was observed difference between the cashew apple (11.33 ± 3.20) and the other treatments. A similar result was found for the courtship patterns in the cashew apple treatment (29.33 ± 4.67), differing from the others, which did not present differences among them. The mating behavior differed between in natura mango and sprayed mango (4.66 ± 1.36), and the treatment with mango (9.66 ± 4.41) and cashew apple (13.50 ± 7.34). The papaya in natura and sprayed papaya did not present difference between the others. On the other hand, to the walking behavior on papaya (23.33 ± 8.26), presented difference among the cashew apple (5.83 ± 3.37), sprayed papaya (7.50 ± 3.44) and sprayed mango (6.00 ± 3.34), not differing to the treatment with mango (16.66 ± 7.39). The other treatments did not present statistical differences among them (Table 2).

Table 2. Comparative analysis of behavioral patterns (mean±SD) of females and males of Ceratitis capitata displayed in 12h light with different oviposition means, by no-choice tests in 25 ± 2 °C temperature and 60% RU environment.

| Behavior Patterns | Cashew | Papaya | Sprayed Papaya | Mango | Sprayed Mango |
|-------------------|--------|--------|----------------|-------|---------------|
| **Females**       |        |        |                |       |               |
| Feeding           | 16.83 ± 5.74<sup>a</sup> | 14.16 ± 7.30<sup>ab</sup> | 6.5 ± 4.54<sup>b</sup> | 13.5 ± 2.88<sup>ab</sup> | 7.83 ± 1.60<sup>b</sup> |
| Water consumption | 11.33 ± 3.20<sup>a</sup> | 6.33 ± 1.63<sup>b</sup> | 2.16 ± 1.47<sup>b</sup> | 6.5 ± 3.20<sup>b</sup> | 5.66 ± 2.80<sup>b</sup> |
| Courtship         | 29.33 ± 4.67<sup>a</sup> | 17.5 ± 2.58<sup>b</sup> | 14.83 ± 6.40<sup>b</sup> | 29.16 ± 4.21<sup>a</sup> | 16.33 ± 5.04<sup>b</sup> |
| Mating            | 13.5 ± 7.34<sup>a</sup> | 9.33 ± 6.02<sup>ab</sup> | 7.16 ± 2.31<sup>ab</sup> | 9.66 ± 4.41<sup>a</sup> | 4.66 ± 1.36<sup>b</sup> |
| Walking           | 5.83 ± 3.37<sup>ab</sup> | 23.33 ± 8.26<sup>a</sup> | 7.5 ± 3.44<sup>ab</sup> | 16.66 ± 7.39<sup>ab</sup> | 6.0 ± 3.34<sup>b</sup> |
| Oviposition       | 0.00 ± 0.00<sup>b</sup> | 6.83 ± 3.12<sup>ab</sup> | 1.33 ± 1.63<sup>b</sup> | 11.16 ± 3.71<sup>a</sup> | 1.5 ± 1.64<sup>bc</sup> |

<sup>1</sup> Groups of shifts (morning and afternoon) which presented difference by the T (P > 0.05).
To the oviposition behavior pattern, the treatment with mango (11.16 ± 3.71) differed from all the other treatments, except the papaya one (6.83 ± 3.12). This only differed from the cashew apple, which was not observed oviposition. The sprayed papaya (1.33 ± 1.63) differed between cashew apple and mango, while the sprayed mango (1.50 ± 1.64) only differed from the in natura mango, not presenting, therefore, significant differences among the other treatments.

About the development of flies in the tested fruits, it was counted 218 adults, with a higher quantity in papaya fruits (97), followed by mango (49), sprayed papaya (48) and sprayed mango (24). No adults were obtained from the cashew apple fruits. After the comparison among the total adults per treatment, papaya treatment differed from the others. The mango fruit treatment did not differ from the sprayed papaya, presenting significant difference (besides the papaya) between the sprayed mango. The sprayed papaya presented difference (besides the papaya) between the sprayed mango.

4. Discussion

The behavioral patterns of fruit flies have been studied with the purpose of helping to control such pests, since they express the daily profile for the carried out activities. On the other hand, These patterns show the times when the flies display certain activities and under what conditions they are displayed, making the control more efficient and rational. These behaviors depend on biotic and abiotic factors, such as the presence of natural enemies, population density, temperature, humidity and host availability, also acting on population regulation [11–13].

The pattern of walking on the fruit is linked to the evaluation of its qualities by the females, which can perform punctures to assess the condition of the fruit, whether or not laying eggs [1,6,14]. There is a reduction in the activities of the females in the treatments with sprayed fruits, suggesting a negative effect on the action of walking and oviposition, showing that papaya and mango fruits in natura presented greater acceptability by females.

In general, insects that have a phytophagous feeding habit with a wider range of hosts, have adaptive advantages, with some hosts considered as primary or preferential, which present better conditions for the development of offspring; however, in the absence of these hosts, others are explored, also called secondary [15]. The oviposition activity is directly linked to the availability of these hosts in the area and also to the type of host (primary or secondary).

It was also noticed a preference of females for the tested exotic fruits (papaya and mango) in natura. The results of the present study show that cashew apples as a non-preferred host for C. capitata, despite having records of development of this fruit in the field (usually with few specimens collected), females avoid laying eggs when exposed only to this fruit; and, when exposed to conditions with choice chance, they preferred exotic hosts [16,17].

For oviposition, there was a preference for fresh mango and papaya fruits, followed by treatments with the same fruits exposed to cashew extract, suggesting that this exposure reduced the attractiveness of the fruits. No oviposition was observed in cashew fruits. The hierarchy of preference of C. capitata for hosts was observed in two distinct populations, with papaya as the preferred host among the tested fruits, followed by mango, orange and apple fruits [18].

Trawling activity was not observed in the present study. However, the occurrence of this phenomenon is directly linked to the oviposition [6]. In natural conditions, two peaks of oviposition of C. capitata were observed: the smallest peak recorded in the early hours

| Activity       | Mean ± Standard Error |
|----------------|-----------------------|
| Courtship      | 36.50 ± 5.95         |
| Mating         | 12.83 ± 7.02         |
| Walking        | 4.33 ± 3.14          |

¹ Means (± DP) followed by the same letters in the line do not differ between them by Tukey test (P > 0.05).

Discussion
of the morning (20%) and the second peak, in the late afternoon (71%) [19]. This activity is related to the release of pheromones which the females use to signal that the fruit is infested, causing other females to seek other hosts to lay their eggs [6]. This behavior is observed in several studies with several species of tefritids, being closely related to the size of the fruit and also to the intra or interspecific competition of larvae [14]. The relationship between the number of offspring and the size of the fruit or local host density is evidenced in several studies e.g., [20–22].

It was observed that males and females showed more frequent activities in the first hours and in the late afternoon, oscillating during the 12 h of light. Rest/inactivity records were more frequent for females than for males. However, in all treatments, this phenomenon represented the largest portion observed for both sexes, with a high difference between the other observed behaviors, not being used for the analyzes. This pattern was noticed by [19] in studies in apple and fig orchards, differing between sex and the place of occurrence (fruit for females and leaves for males). Any activity can be attributed as a response to some stimulus, then the females initially tend to remain inactive until the perception of the stimuli emitted by the males or by the fruits.

In order to carry out oviposition, some barriers must be overcome. The peel is a physical barrier, configuring itself as a limiting factor and a possible primary characteristic in the evaluation of the fruits by the females. This is mainly due to the size of the ovipositor, which may not be large enough to overcome this barrier, characterizing the fruit as not suitable for laying eggs [23]. The fruits studied in the present investigation (papaya, mango and cashew apple) did not have thick skins, so this factor cannot be considered as influencing the results. Among them, mango is the one with the greatest thickness. Nonetheless, it was observed, for this fruit (without the treatment with the cashew extract), the highest levels of oviposition (11.16 ± 3.71), showing that this factor did not determine the occurrence of such activity in the results. Although, the preference of exotic fruits for C. capitata is reported, whereas species of Anastrepha have preference for native fruits, showing coevolutionary adaptations between these species and their hosts [24]. In tests with tropical fruits, using the same strain of C. capitata from the present study, a preference for oviposition was observed in guava (Psidium guajava L.), soursop (Annona muricata L.) and acerola (Malpighia glabra L.) fruits, followed by carambola (Averrhoa carambola L.), Malay apple (Syzygium malaccensis L.), cashew and yellow cajá (Spondias mombin L.). The oviposition behavior was attributed through a preference associated with the nutritional value of the fruit, providing optimal conditions for the development of the offspring [25].

In studies which evaluated the compounds present in cashew, it was possible to observe a large quantity of ethanol, since this fruit undergoes fermentation very quickly. However, when fruit-based baits were tested in the field, no differences were found in the attractiveness between the cashew, hydrolyzed protein, mango and papaya baits for the first two days tested, showing differences between the protein and all others based on fruit on the seventh day of exposure [26,27].

It was noticed that the activity of attracting males preferably occurs in the early morning, showing a reduction in the following hours. It increases again in the late afternoon, differing between shifts for all evaluated treatments. Similar results, with the same fruit flies species, were found in natural environment conditions, presenting two peaks throughout the day: a larger peak in the morning and another in the late afternoon [19,28]. The same phenomenon is registered for Anastrepha fraterculus (Wied., 1830), which, in semi-field conditions, showed higher activity between 07:00 and 11:00, whereas males of A. obliqua preferentially display the activities between 13:00 and 19:00 [29]. In subsequent studies, [30] showed the highest frequency of sexual activity of A. obliqua in the afternoon, with the time between 15h30 and 16h30 as the preferred time for courtship and mating. The daytime behavioral pattern for A. zenildae is also known [31].

From the cashew apples fruits, emergencies were not recorded. These results suggest that cashew does not represent a host with suitable characteristics for females, and the extract sprayed on the exotic fruits, which are its preferred hosts, has a negative influence
on the oviposition behavior, reducing its frequency when compared with fresh fruits. In tests involving the effect of eight tropical fruits on the biology and behavior of *C. capitata*, it was observed that guava, star fruit and soursop fruits showed the best performance of the biological parameters evaluated, with the cycle varying from 14 to 18 days, while fruits of cashew and acerola showed regular development with a cycle of 19 to 20 days [25].

5. Conclusions

Cashew fruits are not attractive to *C. capitata* females, with no record of oviposition and development in the tested conditions. Males display the pattern of attraction preferentially in the early hours of the day, which is not influenced by the available fruit, but the courtship, treated here as the moment of interaction between males and females where there is the choice of mate for copulation, differs between the morning and afternoon shifts and also between treatments with different fruits. Papaya fruits had a higher number of offspring. Cashew extract, when sprayed on fruits that are the preferred hosts for females (papaya and mango), reduces the frequency of total activities, with less oviposition and, consequently, giving rise to fewer adults than fresh fruits.

Author Contributions: R.d.S.B.—analyzed the data. All the other authors—concived and designed the experiments, performed the experiments, wrote the paper. All authors have read and agreed to the published version of the manuscript.

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