

RESEARCH ARTICLE

Innate Host Habitat Preference in the Parasitoid *Diachasmimorpha longicaudata*: Functional Significance and Modifications through Learning

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Abstract

Parasitoids searching for polyphagous herbivores can find their hosts in a variety of habitats. Under this scenario, chemical cues from the host habitat (not related to the host) represent poor indicators of host location. Hence, it is unlikely that naïve females show a strong response to host habitat cues, which would become important only if the parasitoids learn to associate such cues to the host presence. This concept does not consider that habitats can vary in profitability or host nutritional quality, which according to the optimal foraging theory and the preference-performance hypothesis (respectively) could shape the way in which parasitoids make use of chemical cues from the host habitat. We assessed innate preference in the fruit fly parasitoid *Diachasmimorpha longicaudata* among chemical cues from four host habitats (apple, fig, orange and peach) using a Y-tube olfactometer. Contrary to what was predicted, we found a hierarchic pattern of preference. The parasitism rate realized on these fruit species and the weight of the host correlates positively, to some extent, with the preference pattern, whereas preference did not correlate with survival and fecundity of the progeny. As expected for a parasitoid foraging for generalist hosts, habitat preference changed markedly depending on their previous experience and the abundance of hosts. These findings suggest that the pattern of preference for host habitats is attributable to differences in encounter rate and host quality. Host habitat preference seems to be, however, quite plastic and easily modified according to the information obtained during foraging.

Introduction

Host plants are a major source of information for insect parasitoids during host searching [1–3], even when they are not infested [4–6]. The damage caused by the herbivore can induce the plants to release specific compounds (termed herbivore-induced plant volatiles) that increase the attraction of parasitoids [7–9]. Communication between the first and third trophic level is expected to be particularly enhanced when the herbivores are concealed within the host plant, thus reducing to a minimum the available information about their location [10].

The type of chemical cues used by parasitoids during host searching has been related by Vet and Dicke [11] to the range of plant species where the host can be found. When the hosts are generalists, female parasitoids should rely on general cues, common to all possible host plant species, and would follow specific cues only after they acquire experience and associate conspicuous cues (mainly from the plant) to the host presence, through associative learning [12–15]. However, Steidle and van Loon [16] found many examples of parasitoids of generalist hosts that innately respond towards chemical cues from the host habitat. Vet and Dicke's [11] concept does not consider that different host plants species may differ in their profitability (host density and/or encounter rate) as well as in the quality of the hosts they harbour [17–19]. In fact, if host availability differs among host plants, optimal foraging theory [20,21] predicts that females should always prefer the most profitable host plant. Therefore, an innate response towards chemical cues could have evolved from some host-plant systems if the reward in terms of cumulative fitness varies among them [22]. Likewise, if the nutritional quality of the herbivore host varies among plant species, natural selection should favour female parasitoids that are attracted to plants in which the hosts are nutritionally better [23–26], a concept known as the preference–performance hypothesis (PPH) [27,28]. PPH has been widely studied in bi-trophic scenarios: host plant–herbivore (see Gripenberg et al. [28] and references herein) and parasitoid–herbivore host [29–31].

Previous studies have shown that the plant species on which the herbivores develop influences parasitoid's offspring performance [32–35]. Furthermore, there are robust evidences that host plant species affect the second and third trophic levels simultaneously [36–41]. Concurrently, several studies on parasitoids reported a preference for particular host habitats [42–44]. Nonetheless, there is a limited number of studies that simultaneously addressed preference and performance considering the first and third trophic level [45], and are mainly focused on parasitoids associated to Brassicaceae species [45–47] or to Tephritidae fruit fly species [48,49].

Diachasmimorpha longicaudata (Ashmead) is a koinobiont endoparasitoid of Tephritidae larvae. In its habitat of origin, *D. longicaudata* parasitizes larvae of several species of the genus *Bactrocera* [50,51], while they are feeding inside the fruit. Most of its hosts are polyphagous (for instance *Bactrocera dorsalis* Hendel attacks more than 150 fruit species), attacking several unrelated families of plants [51,52], including (Caricaceae, Moraceae, Myrtaceae, Rosaceae, and Solanaceae, among the most important). Because larvae develop entirely in the same fruit, fruit species may be a reliable indicator of the quality of the larvae as hosts. Fruit species could also vary in the host encounter or parasitization rates, so the costs (energy and time allocated to host searching) would differ among host fruits [30]. These differences in potential rewards could have led to host fruit preference. In fact, innate preference for fruit species has been reported for this species [53,54]. Nonetheless, the evolutionary forces that forged these preference patterns have seldom been addressed because [as stated by 45] preference and performance associated to different host habitats have been studied separately [33,55,56] except for Eben et al. [48] and Ovruski et al. [57] who found no preference for fruit species that differentially affected female performance.

Parasitoids of polyphagous hosts, such as *D. longicaudata*, should profit from adjusting their foraging preferences to the distribution of their hosts. Associative learning has been

documented for fruit fly parasitoids [58,59], including *D. longicaudata* [60], however its role in modulating the host habitat preference has not been addressed. Likewise, the fact that the density of host larvae varies among different fruits [61,62] has not been considered.

In this work, we addressed the innate preference of *D. longicaudata* females among odours from four host habitats and, when a preference pattern was found, we tested both the PPH and the potential benefits in terms of parasitization rate. We also tested the hypothesis that *D. longicaudata* is able to adjust host habitat preferences as a function of the density of hosts in the available habitats. Finally, because *D. longicaudata* is capable of associative learning during host finding [60], we hypothesized that the preference for chemical cues of available fruit species is modified by experience to the point where innate preference will no longer drive parasitoid behaviour.

Methods

Insects and fruits

Parasitoids and fruit fly larvae were obtained from the rearing facility at Instituto de Genética “E. A. Favret” (IGEAF) [63]. *Diachasmimorpha longicaudata* colony was initiated with individuals coming from Centro de Investigaciones para la Regulación de Poblaciones de Organismos Nocivos (CIRPON), Argentina [64] in 2001. *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) larvae were used as host. Larvae were reared on artificial rearing medium (a mixture of carrot, sugar, brewer’s yeast, corn flour, and food preservatives, according to Terán [65]) and were exposed to parasitoid females in small Petri dishes when they reached the third instar (following Viscarret et al. [63]). Assays were carried out with 5–7 days-old females with no oviposition experience (except in experiment 3). Females were maintained with males under controlled conditions ($25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ R.H., and 14:10 L:D photoperiod), and were provided with honey and water ad libitum. Females had no contact with fruit or fruit odours until the test. Fruit showing no signs of insect infestation were obtained from the local market. All fruit were thoroughly washed with tap water, measured and maintained at $25 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ R.H. until the tests.

Y-tube olfactometer

The olfactory preferences of *D. longicaudata* were tested using a Y-tube glass olfactometer, in which the females cannot see the fruit and they only perceive chemical cues. The air flow inside the Y-tube was generated by extracting air with a pump (AIR CADET Barnat, USA) connected to the end of the device. The air entered the system through a pair flowmeters (Bruno Schilling, Argentina) that were set at 300 ml/min (0.014 m/s). After entering the device the air was filtered (with glass wool and activated charcoal) and then bubbled in distilled water. Subsequently, the air stream entered two acrylic boxes (3.375 L) where the odour sources were held. Acrylic boxes were connected to the Y-tube which consisted in a two 15 cm-long arms (60° angle between arms, internal diameter 3cm) converging in a 15 cm-long central tube. The distal extreme of the central tube has a hole to introduce the insect. All parts were connected using colourless and odourless plunges and tubing (Dow Corning, Midland, Michigan, USA). The Y-tube assembly was illuminated by a fluorescent light tubes that provided a homogenous illumination of 1200 ± 100 luxes. The room was maintained at $26 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ R.H.

Females were individually released inside the tube and only after they showed signs of acclimatization (i.e., exhibited host searching behaviours such as antennation) the air pump was turned on. Insects were given 10 min to choose an arm in the olfactometer. It was considered that a female made a choice when it walked into one of the arms, surpassed a distance of 4 cm from the end of the central tube and stayed beyond that limit for more than 30 s. The chosen

arm and the latency (time since the release to the decision) were recorded. If no choice was made in 10 min, the assay was concluded.

Every five tested females, the Y-tube and the acrylic boxes were cleaned with hot water, rinsed with ethanol, and left to air dry at room temperature. With each new experimental series of five females, the location of the odour sources was switched in the opposite arrangement. Forty females were evaluated for each treatment.

Experiments

Experiment 1. Preference of *D. longicaudata* females for different fruit species. Four fruit species were tested: peach (*Prunus persica* L., variety Elegant Lady), fig (*Ficus carica* L., variety Brown Turkey), orange [*Citrus sinensis* (L.) Osbeck, variety Valencia], and apple (*Malus domestica* Borkh, variety Red Delicious). These species represent three of the most important taxonomic families where *Bactrocera* larvae can be found in nature [52]. Fruit were offered to female parasitoids in every possible pair-wise combination. Forty females (replicates) were analysed per combination. Each acrylic box contained one apple, one peach, one orange or three figs. This way the amount of surface of each fruit was as similar as possible.

Experiment 2. Effect of the fruit infestation level on females' preference for host habitats. For this set of experiments, apple and orange were selected as study models and female choice was evaluated in four different scenarios: a) orange with high infestation vs. orange with low infestation; b) apple with high infestation vs. apple with low infestation; c) orange with high infestation vs. apple with low infestation; and d) apple with high infestation vs. orange with low infestation. Forty females (replicates) were analysed per combination. This pair of fruits was used as model based on the result of experiment 1 and the fact that are available in the Argentinean market throughout the year (opposite to fig and peach which are available for 1 and 3 months, respectively).

Infested fruit were obtained by placing 60 *C. capitata* females inside a rearing cage (64 L) with four fruit for 1 hour. After infestation, fruit was placed individually in plastic containers (1 L) and kept under controlled conditions ($25 \pm 1^\circ\text{C}$; $40 \pm 5\%$ R.H.). To estimate the infestation level without damaging the fruit, the number of larvae that exited the fruit was recorded the day after the first emerging larvae were detected. If one or two larvae exited the fruit within this 24h time frame, the fruit was assigned to the low infestation level; whereas if 5–10 larvae were recorded the fruit was assigned to the high infestation level. After the assay, the fruit were labelled and kept individually until all larvae emerged and could be counted. Highly infested fruits should have had 30–40 inside at the time of the experiment, whereas fruits with low infestation should have had 5–10 larvae. This procedure allowed obtaining infestation levels that resembled low and high infestation in nature. [66]. Those experimental series (i.e., five females) for which the infestation estimation method was not effective for predicting larval abundance, were removed from the dataset.

Experiment 3. Effect of previous experience on females' preference for host habitats. Host habitat preference was assessed using females that had been offered host larvae associated to a specific habitat during a conditioning period. Again, orange and apple were used as experimental models. Conditioning protocol followed Segura et al. [60], which proved effective to condition *D. longicaudata* females to a visual stimulus. During the conditioning period, 15 females were offered a small Petri dish (5 cm in diameter, 1 cm high, wrapped in *voile* fabric) containing 100 larvae of *C. capitata* immersed in orange or apple pulp for 6h. Exposed larvae were kept under controlled conditions ($25 \pm 1^\circ\text{C}$; $65 \pm 5\%$ R.H.) until adult emergence as to check that females had successfully parasitized them during the conditioning period. The procedure was repeated for three consecutive days. On the fourth day, conditioned females were

used in olfactometry assays where preference for orange or apple was assessed. Forty females (replicates) were analysed per combination.

Experiment 4. Quality of different fruit species as foraging substrates. In order to compare the foraging efficiency of *D. longicaudata* in different host habitats, infested oranges, apples, figs and peaches were exposed to naïve parasitoids. Infestation was achieved as described in experiment 2 (except for fig, in which 12 fruit were exposed to 60 *C. capitata* females). Fruit was then measured and placed in individual containers. When the 3rd larval instar was reached, fruit were individually exposed to *D. longicaudata* females for 6 h. Oranges, apples and peaches were exposed to 5 female parasitoids, whereas figs were exposed to 3 female parasitoids. This proportion allowed standardizing the area offered to the females (ca. 25 cm²/female) among different fruit species. 120 replicates were carried out for each fruit species.

After exposure to parasitoids, fruit were placed in individual containers and kept under controlled conditions (25 ± 1°C; 40 ± 5% R.H.). Pupae were collected, counted, individually weighed to the nearest 0.1 mg using a precision scale (Denver Instrument, NY, USA). Afterwards, pupae were transferred to glass containers and maintained under controlled conditions (26 ± 1°C; 70 ± 10% R.H.) until adult (parasitoids or flies) emergence. The number of flies and parasitoids was recorded and the non-emerged pupae were dissected under a stereomicroscope to determine if the content corresponded to a fly or a parasitoid.

Experiment 5. Quality of host larvae reared on different fruit species. Several bionomic parameters were evaluated on parasitoids emerging from experiment 4. To this end, a female and a male parasitoid emerged from larvae developed in the same fruit species were placed in glass flasks (500 ml) with water and honey, and kept at 22 ± 1°C, 60 ± 5% R.H. and a 14:10 L:D photoperiod.

The mortality was recorded daily. Whenever a male died, it was replaced by another male to ensure continuous availability of sperm. One week after female emergence, 70 3rd instar *C. capitata* larvae, confined in small Petri dishes, were exposed to females for 6h. For each female, 9 larval exposures were performed every 48–72 h covering the period of higher fecundity [63]. Exposed larvae were transferred to a vial containing fresh artificial larval medium which in turn was placed inside a larger container with a layer of vermiculite and kept under controlled conditions (25 ± 1°C; 70 ± 5% R.H.). Every 48h, pupae were collected and counted, and then placed in glass flasks (450 ml). The number of emerged flies and parasitoids (males and females) was recorded. Non emerged pupae were dissected under a stereomicroscope to determine if the content corresponded to a fly or a parasitoid. For apple and orange, 40 couples (replicates) were evaluated, whereas 36 and 30 replicates were carried out for fig and peach, respectively.

Dead females were preserved in ethanol 70%. After all female died, a random sample was taken from each group (28, 32, 18 and 15 for apple, orange, fig and peach respectively) and the right front wing of each female was dissected and placed on a slide over a thin layer of silicone. Wings were photographed under a stereomicroscope (Motic Group Co., China) and the length of the wing was measured afterwards using Motic Images Plus 2.0 software (Motic Group Co., China).

Data analysis

In experiments 1 to 3, a G-test of goodness of fit (with Yates' correction for continuity) was used to compare the frequency of insects visiting each option. Latency was compared between options by means of a Student *t*-test (assumptions were met in all cases).

In experiment 4, parasitism rate was estimated as: (total number of parasitoids / total number of recovered pupae) x 100. For this rate, emerged and non-emerged insects (flies and

parasitoids) were considered. This variable was compared by means of a one-way ANOVA, followed by a post hoc Tukey's multiple comparisons test. As to address whether the different fruit species offered the same possibility of finding host larvae, the number of pupae recovered per fruit per unit area was compared by means of a one-way ANOVA and a post hoc Tukey's multiple comparisons test after the natural log transformation.

In experiment 5, the mean fecundity (number of offspring per female in each exposition), the total number of offspring per female (lifetime fecundity), the sex ratio (number of females in the progeny/ total number of offspring) and female right wing length were compared by means of a one-way ANOVA, followed by a post hoc Tukey's multiple comparisons test. Sex ratio was previously transformed to logit to meet ANOVA assumptions. The weight of *C. capitata* pupae recovered from different fruit species was compared through the estimation of the mean and the confidence intervals. For this variable, severe heteroscedasticity impeded to run an ANOVA or even a Kruskal-Wallis test, as no transformation allowed reducing differences in variance among treatments. Heteroscedasticity was due to a significantly larger variation in weight of pupae recovered from fig, so an exploratory ANOVA (followed by a post hoc Tukey's multiple comparisons test) was carried out with the remaining fruit species.

Longevity was compared among treatments by means of a Cox-Mantel survival analysis. Survival curves were computed using the Kaplan-Meier method.

Tests were performed using STATISTICA for Windows [67].

Results

Experiment 1. Attraction of *D. longicaudata* females' to different fruit species

Females showed a clear preference for figs compared to the other fruit (peach, orange and apple) ($G_{\text{fig-peach}} = 4.30$, $p = 0.038$; $G_{\text{fig-orange}} = 9.40$; $p < 0.01$; $G_{\text{fig-apple}} = 23.42$; $p < 0.01$). Peach was preferred over orange and apple ($G_{\text{peach-orange}} = 5.76$, $p = 0.016$; $G_{\text{peach-apple}} = 14.07$, $p < 0.01$). Finally, females showed a preference for orange over apple ($G_{\text{orange-apple}} = 7.46$, $p < 0.01$). These results showed a hierarchical pattern of female preference for different fruit species. The most attractive fruit was fig, then peach, then orange and finally apple (Fig 1). There were no significant differences in latency between options, except for the fig that was chosen more quickly than the orange (S1 Table).

Experiment 2. Attraction of *D. longicaudata* females' to fruit with different levels of infestation

When fruit of the same species were offered to the females in the Y-tube, a clear preference for the most infested fruit was shown ($G_{\text{apple high-apple low}} = 14.32$, $p < 0.01$; $G_{\text{orange high-orange low}} = 7.46$, $p < 0.01$) (Fig 2). Preference for the most infested fruit was also found when females were offered an orange with high infestation and an apple with low infestation ($G_{\text{apple low-orange high}} = 11.59$, $p < 0.01$) (Fig 2). However, apples with high levels of infestation were not more attractive than oranges with low levels of infestation ($G_{\text{apple high-orange low}} = 0.63$, $p = 0.428$) (Fig 2). Latency times did not differ between treatments (S2 Table).

Experiment 3. Effect of previous experience on *D. longicaudata* females' preference

In the Y-tube olfactometer, a significantly higher proportion of females oriented towards the fruit on which they were conditioned, irrespectively of the fruit species (Females conditioned on orange: $G_{\text{apple -orange}} = 16.85$, $p < 0.01$; females conditioned on apple: $G_{\text{apple -orange}} = 5.76$,

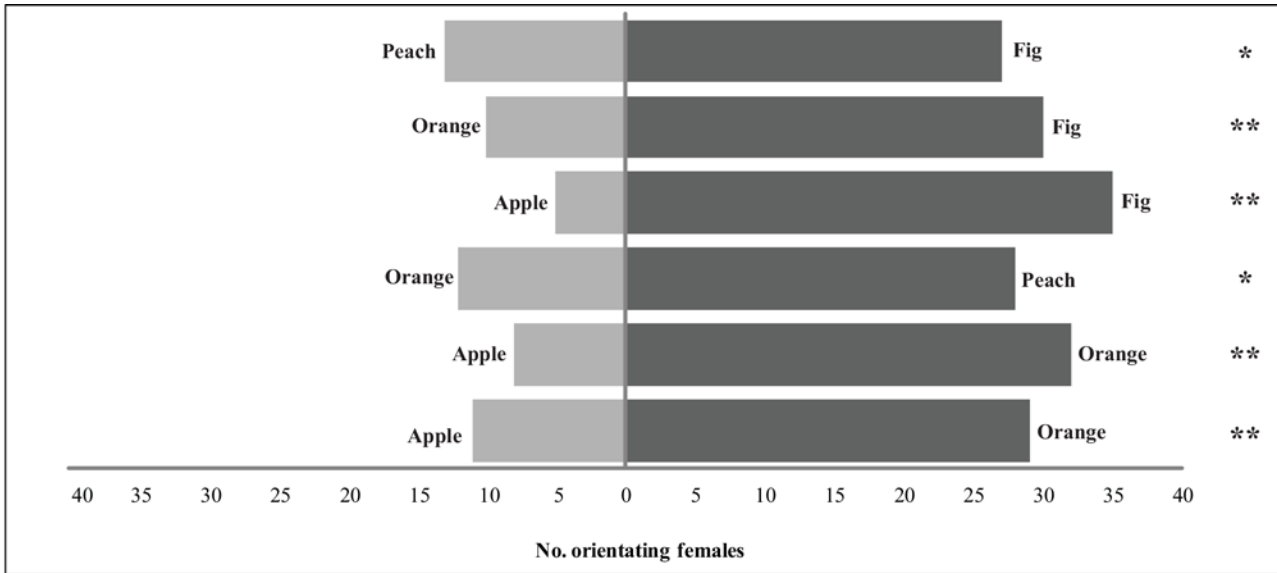


Fig 1. Preference for different fruit species by *Diachasmimorpha longicaudata* females in a Y-tube olfactometer. Bars show the number of females visiting each option in each pair-wise combination among the four fruit species (experiment 1). G-test level of significance: * $p < 0.05$; ** $p < 0.01$.

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$p = 0.016$) (Fig 3). Latency times did not differ between treatments (S3 Table). Parasitism controls showed that all the females used in experiment 3 had successfully parasitized larvae during the conditioning period.

Experiment 4. Quality of different fruit species as foraging substrates

While the number of fruit exposed to parasitoids was 120 for each fruit species, several replicates were lost for different reasons. In some cases, larvae were not recovered from some fruit that were considered to be infested (and were consequently offered to parasitoids). Likewise, some fruit were colonized by fungi and larval survival was severely affected. These cases were

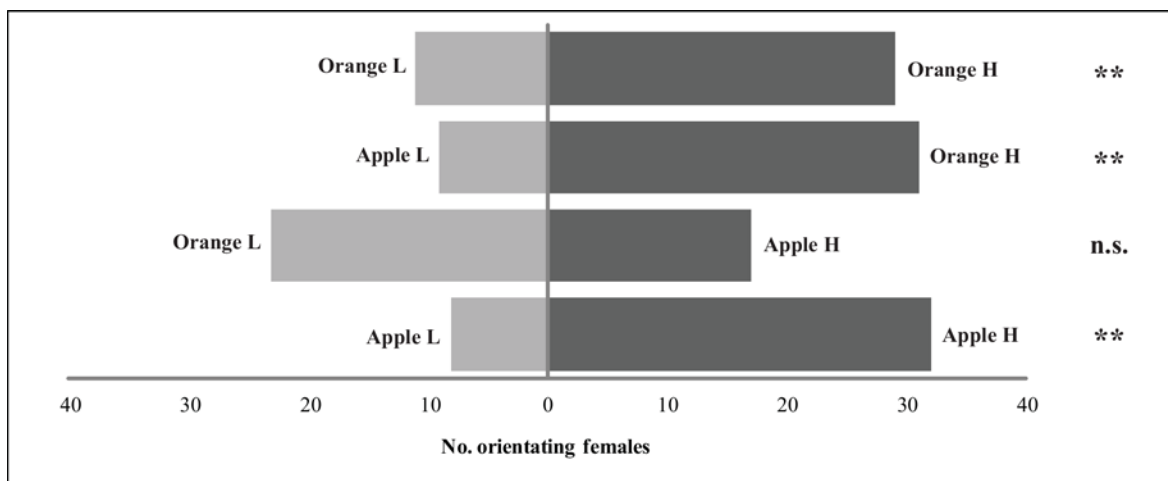


Fig 2. Preference of *Diachasmimorpha longicaudata* females for fruit with different levels of infestation by *Ceratitidis capitata*. Bars show the number of females visiting each option in the experiment 2. H: high infestation level; L: low infestation level. G-test level of significance: n.s. = non-significant; * $p < 0.05$; ** $p < 0.01$. n.s.

doi:10.1371/journal.pone.0152222.g002

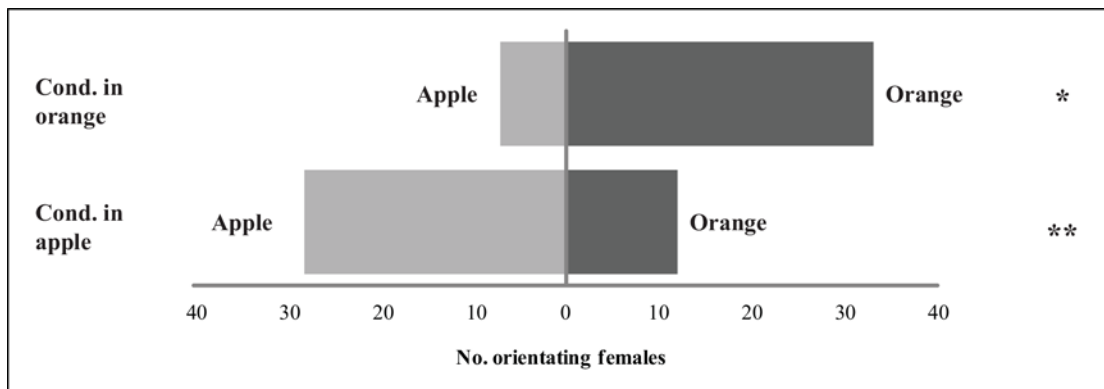


Fig 3. Effect of previous experience on the preference of *Diachasmimorpha longicaudata* females for different fruit species. Bars show the number of females (conditioned either on apple or orange) visiting each option in the experiment 3. G-test level of significance: * $p < 0.05$; ** $p < 0.01$.

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not included in the dataset and consequently the number of replicates was 295 (Apple: 58 fruit; Fig: 58 fruit; Orange: 64 fruit; Peach: 115 fruit).

The parasitism rate ranged between 16.76% and 55.66%, and significantly differed among fruit species (ANOVA: $F_{3, 291} = 28.43$; $p < 0.01$). Tukey's multiple comparisons showed that the parasitism rates in fig were higher than in apple, orange and peach (Fig 4A). In turn, apple showed higher rates than orange and peach, which showed no differences between them (Fig 4A).

The number of larvae per cm^2 of fruit surface offered to female parasitoids was significantly different among fruit species (ANOVA: $F_{3, 291} = 22.74$; $p < 0.01$) (Fig 4B). Multiple comparisons post hoc test showed that apple had significantly lower density of larvae than the other fruit species (Fig 4B), whereas no differences were found among the other three species (Fig 4B).

Experiment 5. Quality of host larvae reared on different fruit species

Mean daily fecundity ranged between 13.14 and 19.41 parasitoids/female and the total fecundity ranged between 106.13 and 160.12 parasitoids/female. Significant differences were found in the mean fecundity of parasitoid emerged from larvae that had fed on different fruit species (ANOVA: $F_{3, 142} = 32.08$, $p < 0.01$) (Table 1). The mean fecundity of females emerged from larvae reared on oranges was higher than the other fruit species, which in turn did not differ statistically (Table 1). The same pattern was observed for the lifetime fecundity (ANOVA: $F_{3, 142} = 31.80$, $p < 0.01$; Table 1). The sex ratio differed significantly among treatments (ANOVA: $F_{3, 142} = 6.26$, $p < 0.01$). Females reared on larvae that developed in oranges showed a higher proportion of females in the progeny than females reared in the other three fruit species, which showed no differences among them (Table 1). Survival analyses showed no differences in lifespan among treatments, both for females and males (Cox-Mantel test: Males: $\chi^2_3 = 2.81$, $p = 0.421$; Females: $\chi^2_3 = 6.28$, $p = 0.099$) (Table 1, Fig 5).

The weight of pupae reared in each fruit species was higher for peach and orange; fig showed intermediate values, and apple showed the lowest values (Table 2). The differences among the four fruit species could not be analysed statistically, and the ANOVA performed excluding the fig showed significant differences among the other three species (ANOVA: $F_{2, 234} = 54.78$, $p < 0.01$). Post hoc comparisons showed that pupae that developed in apple were lighter than pupae that developed in orange and peach, between which there were no significant differences in weight (Table 2).

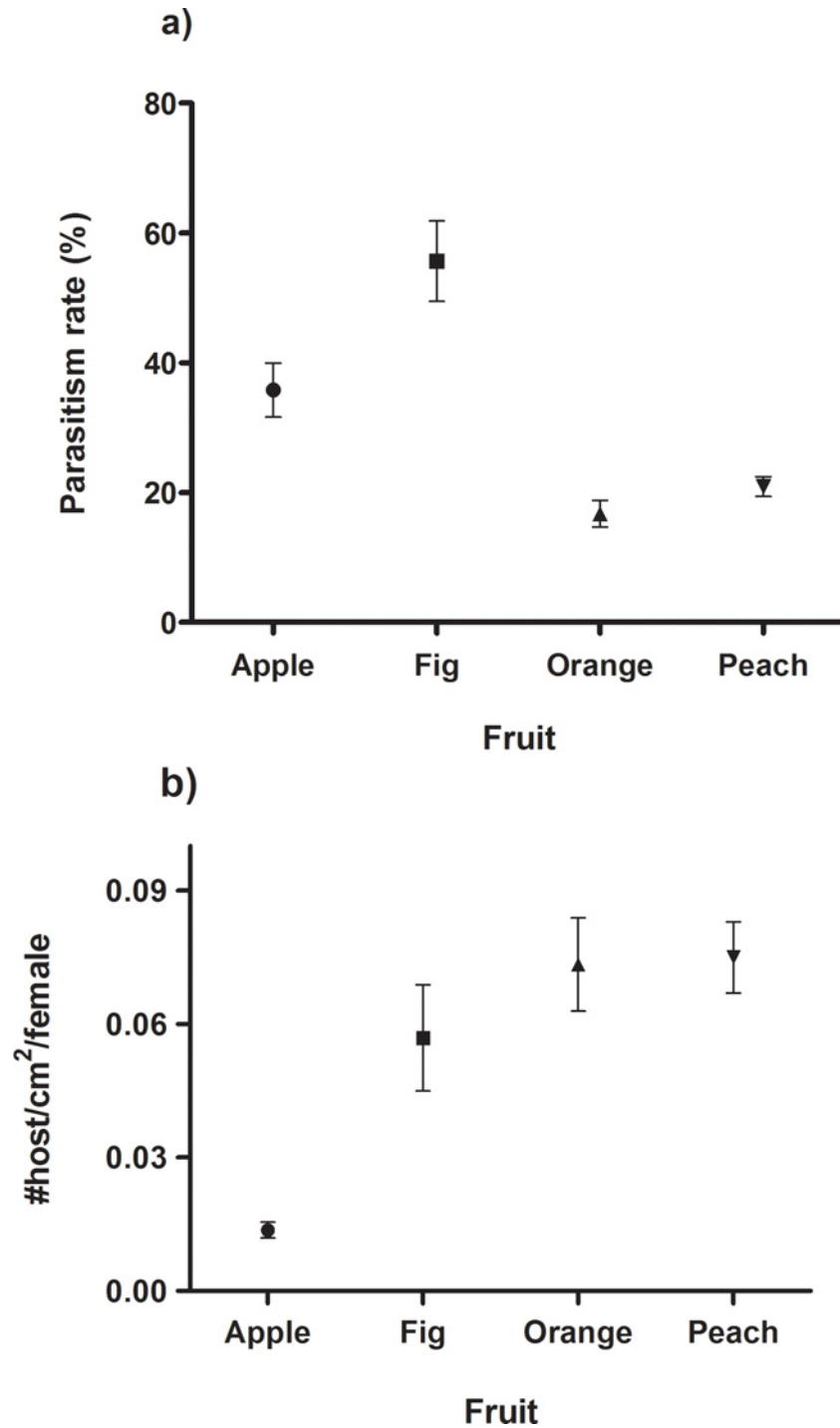


Fig 4. Quality of different fruit species as foraging substrates. a) Mean parasitism rate by *Diachasmimorpha longicaudata* recorded in different fruit species infested by *Ceratitis capitata* larvae. b) Mean density of larvae offered during infested fruit exposure in experiment 4. Bars show the standard error of the mean.

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Wing length was statistically different among females emerged from larvae reared in different fruit species (ANOVA: $F_{3, 89} = 5.37$, $p < 0.01$). Tukey's test showed that wings from females

Table 1. Mean fecundity (mean ± S.E.), total fecundity (mean ± S.E.), sex ratio (mean ± S.E.) and lifespan (mean ± S.E., in days) of *Diachasmimorpha longicaudata* reared in fruit fly larvae fed on different fruit species in experiment 5.

Fruit species	Mean fecundity	Total fecundity	Sex ratio	Female lifespan	Male lifespan
Apple	13.14 ± 1.03 a	106.13 ± 10.11 a	0.477 ± 0.019a	41.25 ± 2.62	26.56 ± 1.94
Fig	15.89 ± 1.23 a	128.89 ± 10.87 a	0.500 ± 0.026a	35.19 ± 2.56	27.50 ± 1.78
Orange	19.41 ± 1.11 b	160.12 ± 13.79 b	0.646 ± 0.021b	35.17 ± 2.03	24.08 ± 1.64
Peach	14.18 ± 0.90 a	125.18 ± 10.68 a	0.531 ± 0.029a	39.32 ± 2.04	26.80 ± 2.42

Means followed by the same letter within the same column do not differ statistically ($\alpha = 0.05$; multiple contrasts).

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associated to oranges were larger than those associated to figs ($p = 0.017$) and apples ($p < 0.01$), but no differences were found among figs, apples and peaches ($p > 0.05$) (Fig 6).

Discussion

Innate preference for host habitats in parasitoids is expected to occur only if available habitats vary in profitability and/or host quality. In our study, naive *D. longicaudata* females showed a hierarchical preference pattern among non-infested fruit of four different species. This result can be partially explained by a differential reward in terms of fitness associated to each fruit species. Evidence show that the pattern of preference is not fixed and can be modified according to external factors, such as host density, and internal factors, such as experience.

Females preferred fig over the rest of the fruit species, then peach, orange and, finally, apple. This innate preference was not expected to be so strong for a parasitoid which hosts are so polyphagous. The innate response could be related, at least to some extent, to a differences in host density among different fruit species in nature [64,68,69]. Field and laboratory studies showed that the most frequent host fruit for *Bactrocera* species are mango (*Mangifera indica* L.), papaya (*Carica papaya* L.), guava (*Psidium guajava* L.), several species of *Cucurbita*, as well as native species of varying taxonomic families (e.g. *Psidium* sp., *Ziziphus* sp., *Syzygium* sp., etc.). Infestation of *Citrus* spp. by *Bactrocera* is rare [51,70,71]. Moreover, a survey performed of fruit flies (Diptera: Tephritidae) from wild and cultivated host plants species in Thailand and Malaysia found that *D. longicaudata* was frequently recovered from several species of Moraceae family (the same family of fig) [51]. According to White and Elson-Harris [52], apple is not an important host for *Bactrocera* spp. and only 10% of the species of this genus attack this fruit species. Although it is not possible to directly link these finding to the innate preference of *D. longicaudata* found in our experiments, a differential distribution of the native hosts among fruit species could account for the preference for fig over apple and orange.

The innate preference of *D. longicaudata* could also be related to differences among fruit species in the available enemy-free space [72]. Parasitism rates on fig were higher than those found on peach and orange. This might be related to the smaller size of figs which would disable larvae to escape from parasitism [73,74]. These differences could therefore explain the preference of *D. longicaudata* for fig within the olfactometer, but on the other hand no preference should be expected between peach and orange (on which the parasitism rate was not different), and olfactometer results showed a clear preference for peach. (Comparisons of parasitism rates between apple and the rest of the fruit species are limited because of its lower number of larvae per cm²). The association between preference and realized parasitism rate has been studied in *D. longicaudata* by Leyva et al. [53] and Ovruski et al. [57]. Altogether, results so far seem to indicate no clear association between the preferred host fruit and the parasitism rate, at least not an association that completely explains the preference for host habitats.

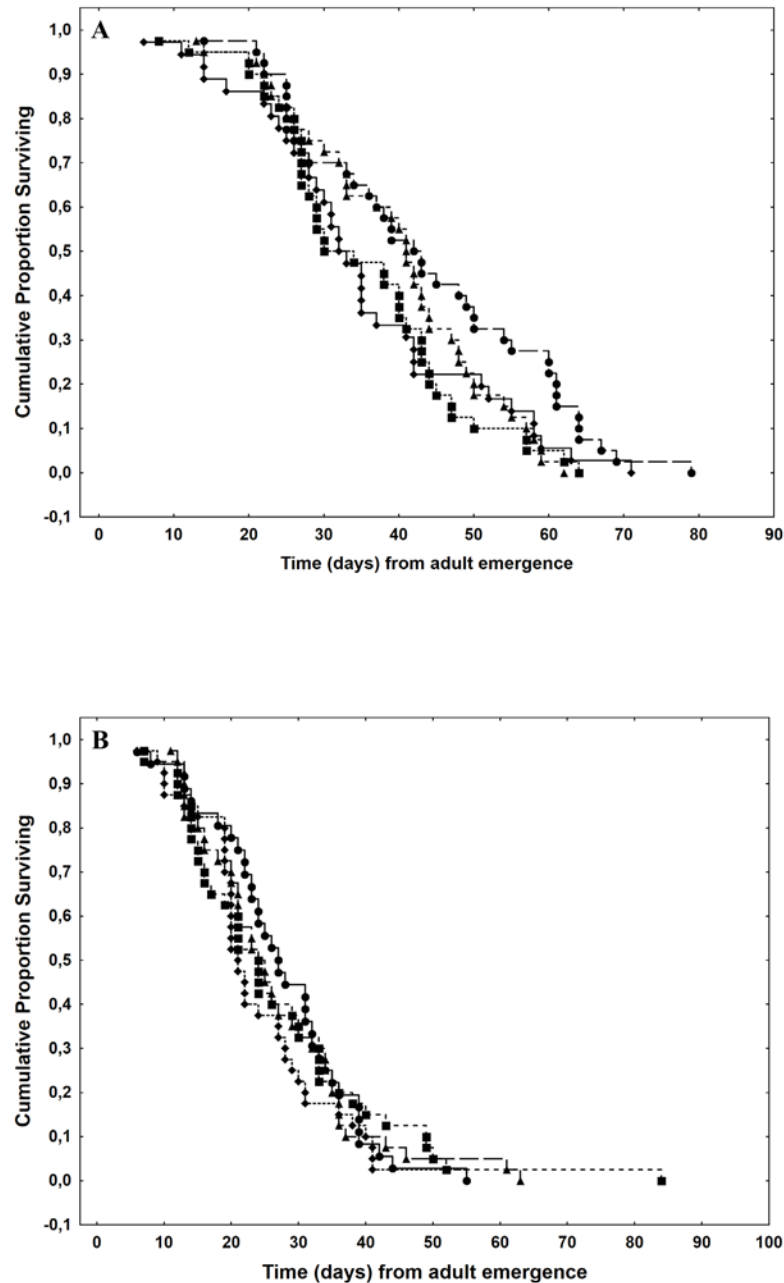


Fig 5. Survival curves for adult *Diachasmimorpha longicaudata* reared on *Ceratitis capitata* larvae that developed in different host fruit. Different fruit species are labelled with a different shape: Apple: circles; Fig: squares; Orange: triangles; Peach: diamonds. (a) males and (b) females.

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Several studies have focused on the PPH at the bitrophic scale, but the connection between preference and performance considering the first and third trophic level has seldom been studied [45]. In the present study, we did not detect a clear association between the preferences of female parasitoids for certain host habitats and their reward in terms of fitness. In fact fecundity and sex ratio showed that performance was higher for parasitoids associated to oranges, while survival was not affected by fruit species. This is in agreement with results obtained by Eben et al. [48] (who compared oranges and mangoes), which together with our results suggest

Table 2. Pupal weight (mean \pm S.E.) of *Ceratitis capitata* reared on different fruit species.

Fruit species	Pupal weight (mg)	CI 95%
Apple	7.09 \pm 0.17a	6.74–7.43
Fig	8.46 \pm 0.36	7.74–9.19
Orange	9.16 \pm 0.15b	8.86–9.45
Peach	9.34 \pm 0.14b	9.06–9.62

Confidence intervals of 95% (CI 95%) are also presented.

Means followed by the same letter within the same column do not differ statistically ($\alpha = 0.05$; multiple contrasts). Fig was not considered in the statistical analysis because of the larger variation around the mean.

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that orange would be a fruit that produces high quality hosts even when it is not the preferred host habitat. This lack of a perfect association between preference and performance when the first and third trophic levels are considered seems to be quite frequent [46,48,53,75] with cases in which PPH is supported being rare [45]. It could be argued that the correspondence between preference and performance fades away as the distance between trophic levels increases, and other factors, such as realized parasitism rate and the oviposition preference of the adult flies, might contribute to a larger extent to the parasitoid preference. This idea needs further research.

The size of the herbivore is a good estimator of its quality as host for koinobiont parasitoids that attack late instar larvae, like *D. longicaudata* [76–80]. We found an effect of the host fruit on the weight of *C. capitata* pupae, with apple showing the lowest weight. Similar results were obtained for adult size, which is considered an important component of parasitoid fitness [18,19, 81]. This could explain the fact that apple was the least chosen fruit within the olfactometer. Nonetheless, these parameters cannot entirely explain the pattern of preference for different fruits, because pupae that developed in peach and orange showed similar weights and adult size, with fig taking intermediate values. Again, preference for host habitats and performance associated to each one of them are not clearly correlated.

Despite an innate preference for host habitats, we found that *D. longicaudata* preference is modulated by the level of infestation (patch richness) and the experience acquired during foraging. *Diachasmimorpha longicaudata* is able to discriminate between infested and non-infested fruit based on infochemicals [48,82–84]. Our results showed that females are also capable of discerning between different levels of infestations, and orientate themselves to the more profitable patches. Moreover, chemical information about host abundance overrides the preference for a given fruit. This suggests that when females have a reliable cue of the host presence, orientation behaviour will no longer respond to innate preferences, something that has been documented in other host-parasitoid systems [13,85–87]. By doing so, parasitoids can adjust their host searching strategy and seek those habitats in which the probability of host encounter is higher.

Previous experience also affected the host habitat preference of *D. longicaudata*. This shows again that *D. longicaudata* can assimilate information about the distribution of its hosts and modify accordingly their foraging behaviour, in this case probably mediated by associative learning (because in the olfactometer trials fruit was not infested). Learning of chemical cues by insect parasitoids has been reported in several species and behavioural contexts, such as food [88–90] or host finding [14,91,92]. The effect of learning is so drastic for this species that it generates a preference for colours when there is no innate preference for such cue [60] and, as shown here, blurs the innate pattern of preference for fruit species based on chemical cues.

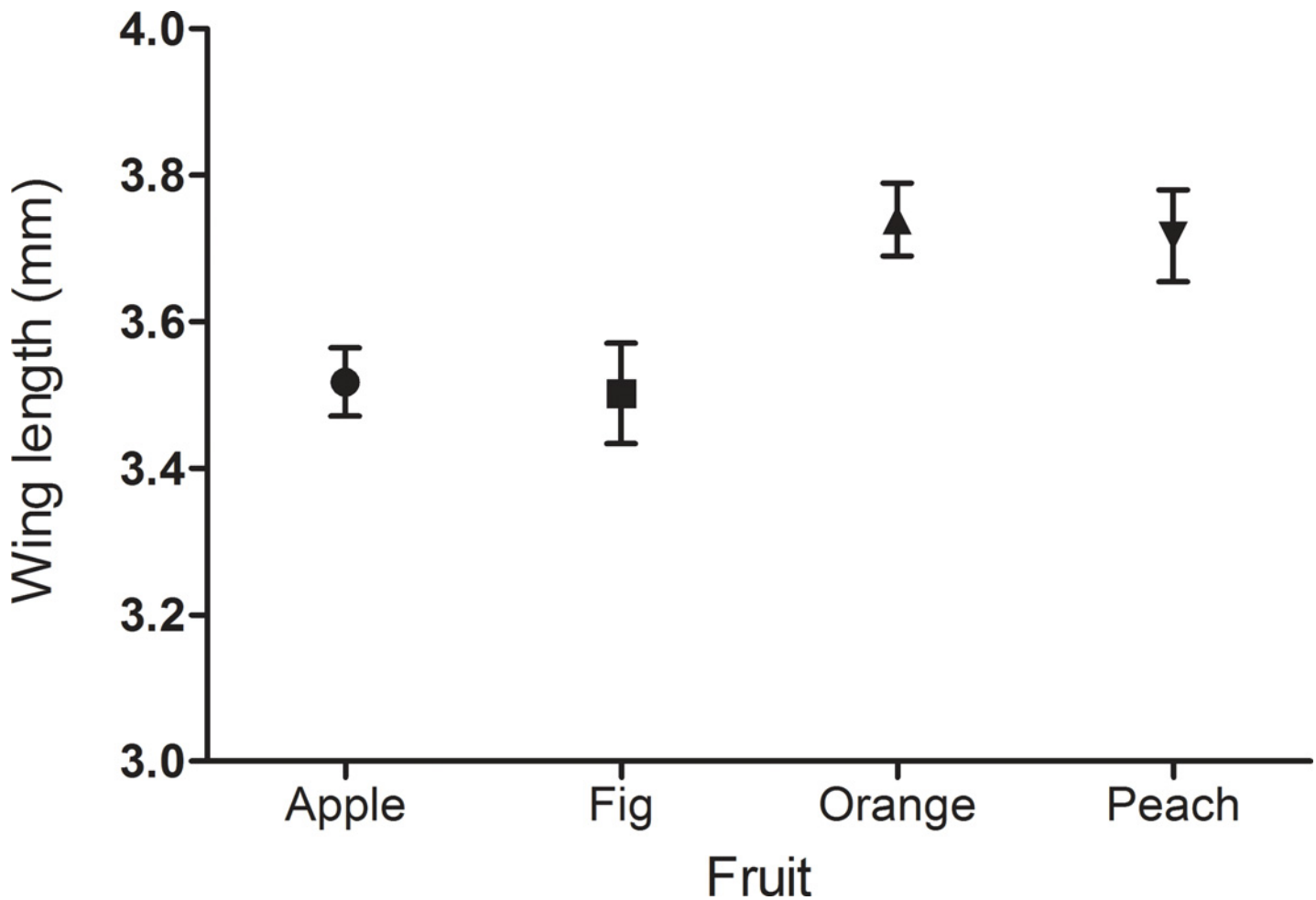


Fig 6. Effect of fruit species on parasitoid size. Mean wing length of *Diachasmimorpha longicaudata* reared on *Ceratitidis capitata* larvae that developed in different host fruit. Bars show the standard error of the mean.

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Our results provide information about the host finding behaviour and, consequently, the effectiveness of *D. longicaudata* as a biological control agent of Tephritidae fruit fly pests. We demonstrated that female parasitoids can use chemical information both from the host and the host habitat during the host searching process. Females will orientate preferentially to specific host habitats in the absence of direct cues from their hosts, which would allow to reduce the searchable area and increases the probability of host encounter. However, if direct cues are perceived, females will orientate to the infested fruits [83] apparently in a dose-response manner, as they are able to detect different infestation levels. After finding suitable hosts, females are able to associate conspicuous chemical and visual cues from these host habitats that can be used in successive foraging bouts. The plasticity in the use of chemical and visual cues makes this species a good candidate to control hosts that can be encountered in fruits of different species, which are ephemeral habitats that change markedly in their chemical and physical properties along the season.

Supporting Information

S1 Table. Latency times (mean \pm S.E.) recorded in the Y-tube olfactometer in experiment 1. (DOCX)

S2 Table. Latency times (mean \pm S.E.) recorded in the Y-tube olfactometer in experiment 2.
(DOCX)

S3 Table. Latency times (mean \pm S.E.) for the selection in the Y-tube olfactometer in experiment 3.
(DOCX)

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Author Contributions

Conceived and designed the experiments: DFS FD MMV SMO JC JLC. Performed the experiments: DFS ALN MMV FD GEB. Analyzed the data: DFS GEB ALN MMV FD JC. Contributed reagents/materials/analysis tools: DFS SMO JC JLC. Wrote the paper: DFS GEB ALN FD MMV SMO JC JLC.

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