Induced Preference Improves Offspring Fitness in a Phytopathogen Vector

D. G. Stockton,¹ L. E. Pescitelli,¹ T. A. Ebert,¹ X. Martini,² and L. L. Stelinski¹

¹Department of Entomology and Nematology, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, ²Department of Entomology and Nematology, North Florida Research and Education Center, University of Florida, Quincy, FL 32351, and ³Corresponding author, e-mail: dara.stockton@ufl.edu

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Abstract

In the Asian citrus psyllid (Diaphorina citri Kuwayama), learning facilitates host recognition and mate preference; however, it is unclear whether induced female oviposition preference occurs in this species. We investigated the influence of natal host experience on adult oviposition preference when reared on either ‘Valencia’ orange (Citrus x sinensis) or orange jasmine (Murraya paniculata). Psyllids reared on ‘Valencia’ orange preferred ‘Valencia’ orange as an oviposition host compared with orange jasmine, whereas there was no difference in oviposition between the two hosts in orange jasmine reared psyllids. Nymphs transferred from ‘Valencia’ orange to orange jasmine were smaller in adult size and required more time for development. These findings were reversed in orange jasmine reared psyllids, which increased in size and displayed shorter development times when transferred from orange jasmine to ‘Valencia’ orange. However, mortality increased in nymphs transferred to the non-natal host species in both treatment groups compared with nymphs transferred to the same host. These results indicate an association between host plant preference and performance in this species. Maternal host experience appeared to influence the oviposition preference in this species. Juvenile psyllid performance appeared negatively affected by orange jasmine plants such that fitness was reduced, suggesting benefits for maternal host fidelity in those insects not acclimated to feeding on orange jasmine. Induced oviposition preference may provide an important mechanism of adaptive plasticity in D. citri reproductive strategies, allowing females to discriminate among potential host species in favor of those to which her offspring are best adapted.

Key words: Asian citrus psyllid, huanglongbing, adaptive plasticity, induced preference and performance, host switching

Phenotypic plasticity allows organisms to adapt to the environment (Agrawal 2001). This can occur as polymorphisms in physical appearance, physiology, or behavioral variation within a population. For example, in response to chemical cues produced by predaceous fish, the water flea Daphnia lumholtzi (G.O. Sars) develops defensive exoskeletal spines, whereas those without exposure do not (Green 1967). Although the capacity to develop a trait may be genetically fixed within a population, expression of those features depends on the environment in which the organism develops. In contrast to physical modification, behavioral plasticity occurs when organisms demonstrate varied behavioral responses to a stimulus based on environmental change (Keiser and Mondor 2013) or past experiences (Menzel 1985). In some cases, when the latter is responsible for such change, that plasticity can be attributed to learning.

Many well-documented examples of behavioral plasticity demonstrate adaptive significance, such that past experience modifies future behavior to improve reproductive success and offspring fitness. In the fruit fly Drosophila melanogaster Meigen, female mate choices may reflect early experiences she has with courting males, where a preference for male size is determined by the courtship encounters early in her adult development (Dukas 2005a). Similarly, male fruit flies appear to use failed courtship attempts to refine their selection of conspecific females and effectively avoid heterospecific species (Dukas 2005b).

In addition to mate choice and courtship behaviors, learning may influence other areas of reproduction, such as oviposition rate and substrate selection (Papaj and Prokopy 1989). Depending on maternal diet and natal host experience, offspring may demonstrate differential host preference and compatibility (Stoyenoff et al. 1994). Indeed, many female insects prefer to oviposit onto plants similar to those of their natal environment to avoid the risks associated with a novel environment (Jaenike 1983, Papaj and Prokopy 1986, Singer et al. 1988, Agrawal et al. 2002). Such preference for plants similar to the maternal natal host appears to directly benefit the offspring. When forced to feed on a novel host plant species with which they are not acclimated, some arthropods show significant declines in performance, such as decreased oviposition, longevity, and size associated with inadequate detoxification of defensive allelochemicals.
Maternal Setting and Oviposition Preference

To investigate the effects of juvenile host species experience on adult female host preference, females reared on either orange jasmine or ‘Valencia’ orange were assayed in a two-choice test to determine settling ($n_{settling} = 40$ per treatment) and oviposition ($n_{oviposition} = 30$ per treatment) preference on flush clippings of both species. Five females and five male psyllids were released per cage. The number of psyllids settling on each clipping was recorded at 24, 48, and 72 h. At 72 h, the number of eggs laid was counted with a dissecting microscope (Leica M50, Leica Microsystems INC., Buffalo Grove, IL).

To avoid cavitation and subsequent wilting, leaf flush shoots were clipped from plants underwater and immediately transferred to individual 20 ml plastic vials (filled with a 2.5 % sucrose solution). One clipping of ‘Valencia’ flush was paired with one clipping of orange jasmine flush, comparable in size (~6 cm) and amount of leaf tissue (~1 g), and placed in a clear, plastic cylindrical cage. The cages measured 28 × 10 cm (height × diameter) and were designed with a mesh top to allow ventilation. Psyllids were confined to the upper 14 cm of each cage using a cardstock divider. This facilitated easy observation of the insects within the cages and caused the insects to move onto the flush clippings.

The settling experiment was replicated seven times with 12 cages (six ‘Valencia’ reared; six orange jasmine reared) per replicate. Of those 12 samples per replicate, six contained psyllids reared on ‘Valencia’ orange and six contained psyllids reared on orange jasmine. After removing four samples (two ‘Valencia’ treatment cages; two orange jasmine treatment cages) due to flush decline in the first replicate, data were collected from 80 cages in total. The oviposition data were collected from the final five replicates because our initial observations did not include egg counts.

Effect of Maternal Host Preference on Nymphal Development

To determine whether maternal host preferences were associated with fitness benefits for the offspring, we transferred nymphs from the maternal host to either the alternative host, or a new maternal host plant; and measured the development time, adult offspring size, the activity level of adult offspring, and nymph mortality. There were four treatment groups: 1) orange jasmine psyllids moved to orange jasmine; 2) orange jasmine psyllids moved to ‘Valencia’ orange; 3) ‘Valencia’ psyllids moved to ‘Valencia’ orange; and 4) ‘Valencia’ psyllids moved to orange jasmine.

We observed the development time (days) of juvenile psyllids from first instar nymphs to adults. First instar nymphs were transferred in groups of 100 to new plants. Three replicates were conducted. Clippings from the maternal host plant species were inspected under magnification and all nymphs other than the first instar stage were removed manually. Thereafter, the clippings were secured to the new transfer plant such that the flush from the clipping was in contact with intact flush on the transfer plant. The clipping was removed after 24 h. This duration was sufficient to allow the nymphs to move independently from the clipping to the new plant. The development time, from the first instar stage at the time of transfer to adult eclosion, was observed daily. Using those data, we calculated the mean development time of from the first instar to adult in each of the four treatments. We also calculated the cumulative emergence of psyllids depending on treatment. We assumed some variation in emergence times because D. citri requires approximately 1.5 d to mature from the first instar to second instar nymphs at 28° C (Lui and Tsai 2000).

Effects of Maternal Host Preference on Adult Offspring Size

After the adults emerged, we removed those psyllids from the rearing cages and stored them in 95% ethanol at -20°C. Then we measured each individual according to five body features with the aid of dissecting microscope at 10x magnification with a 12 mm ocular ruler. Body length, abdominal length, wing length, femur length, and head width were measured. Three replicates of 30–50 nymphs were conducted across the four treatment groups: Orange jasmine → Orange jasmine ($n = 155$; 77F:78M); Orange jasmine → Valencia ($n = 158$; 79F:78M); Valencia → Orange jasmine ($n = 156$; 85F:78M); Valencia → Valencia ($n = 157$; 80F:78M).
Effect of Maternal Host Preference on Adult Offspring Behavior

Those adults not used for morphometric analysis were assayed for activity level to measure the behavior of the offspring. We quantified whether the treatments affected latency to behavioral response in a Y-maze olfactometer, a commonly employed behavioral assay in psyllid research (Mann et al. 2011, Stockton et al. 2017). Virgin 3–7-d-old female psyllids (n = 40 per treatment) were released into the base of the Y-maze and were given a maximum of 5 min to reach a target arm. We defined selection as entry into a Y-maze arm, at least 1 cm past the crux, for a minimum of 30 s. Both arms contained a stimulatory odor target, 0.01 ng/ml E-β-caryophyllene (Sigma-Aldrich, St. Louis, MO; 98% purity) pumped at a rate of 0.5 liter/min using an ARS Inc. (Gainesville, FL) flowmeter with charcoal filters and humidified air. The time to reach a target arm, or the failure to leave the inlet arm, were recorded. The Y-maze olfactometer was replaced with a clean apparatus every five trials. Every 10 trials, the treatment position was switched to control for possible orientation towards non-target environmental stimuli.

Effect of Maternal Host Preference on Nymph Survival

To determine differences in mortality, we performed an additional experiment. In the previous experiment, the first instar nymphs were transferred to new plants passively by placing a clipping on the transfer plant and allowing the nymphs to move independently. This was necessary because attempts to move the first instar psyllids manually resulted in high mortality. At this stage, the insects are extremely delicate and do not tolerate handling. However, because we could not precisely control the number of insects that successfully transferred to the new plant, we were unable to accurately determine mortality. Therefore, direct transfer of a precise number of insects was necessary. We modified the method used previously and transferred the third instar nymphs according to the four treatments previously described: Orange jasmine → Orange jasmine (n = 40); Orange jasmine → Valencia (n = 40); Valencia → Orange jasmine (n = 40); and Valencia → Valencia (n = 40). Two trials were performed per treatment. In each replicate, the mortality of 20 nymphs per treatment was recorded. Survival of the insects was monitored daily. Survival was calculated as the total number of nymphs transferred minus the total number of adult psyllids that subsequently emerged.

Statistical Analysis

Differences in D. citri settling were analyzed with sequential paired t-tests for each time point. We applied a Bonferroni correction to the P-values to reduce the likelihood of Type I error (adjusted α = 0.0167). Oviposition data were analyzed with binomial GLM and non-parametric Wilcoxon sign-rank tests because of the non-normal distribution of the data. Differences in the development time among the four nymph treatments were analyzed with Kruskal–Wallis and Wilcoxon sum-rank tests. Differences in morphometric characters among the four treatment groups were compared with multivariate analysis of variance (MANOVA) and t-tests. We compared the latency to selection among treatment groups with ANOVA. Mortality among nymphs from each treatment was compared with chi-squared tests. Analyses were performed in R 3.0.2 (Version 3.0.2; the R Foundation for statistical software R; Vienna, Austria).

Results

Maternal Settling and Oviposition Preference

We observed differences in settling frequency among psyllids reared on either orange jasmine plants or ‘Valencia’ orange (Fig. 1A and B). Psyllids reared on orange jasmine plants showed no preference at 24 and 48 h post-release, but significantly increased preference for orange jasmine plants at 72 h (paired t-test: t₉ = 2.71, P = 0.009; Fig. 1A). Psyllids reared on ‘Valencia’ orange initially preferred to

Fig. 1. Diaphorina citri settling (A and B) and oviposition (C) choices on host plants based on previous experience. (A) Settling preference among psyllids initially reared on orange jasmine plants (n = 40) and (B) psyllids initially reared on ‘Valencia’ orange plants (n = 40) are indicated by asterisks at each time point (paired t-tests: *≤0.05, **≤0.01, “NS” = non-significant). (C) Differences in oviposition rate (Mean ± SEM) in a two-choice test (orange jasmine flush = white bars; ‘Valencia’ orange = gray bars) among female psyllids reared on either orange jasmine (n = 30) or ‘Valencia’ orange (n = 30; Wilcoxon sign-rank tests: *≤0.05, **≤0.01, “NS” = non-significant).
settle upon ‘Valencia’ plants rather than orange jasmine (paired t-test: \( t_{39} = 3.3, P = 0.002 \)); however, that preference was no longer statistically significant at 48 and 72 h (Fig. 1B).

We also observed differences in oviposition preference depending on host plant experience (GLM with binomial distribution: \( \chi^2 = 182.83, P < 0.001 \); Fig. 1C). Females reared on ‘Valencia’ orange plants (Wilcoxon sign-rank test: \( V = 306, P = 0.005 \)) laid significantly more eggs on ‘Valencia’ flush than on orange jasmine flush; whereas, there were no statistical differences in oviposition rate between the two host species when psyllids were reared on orange jasmine (Wilcoxon sign-rank test: \( V = 279, P = 0.085 \)).

**Effects of Maternal Host Preference on Nymphal Development**

The development time from the first instar nymphs to adult psyllids varied significantly depending on the natal host species and host transfer treatment (Kruskal–Wallis rank-sum test: \( \chi^2 = 113.1, P < 0.0001 \); Fig. 2A and B; Tables 1 and 2). Of the four treatment groups, ‘Valencia’ → ‘Valencia’ psyllids developed 1–2 d faster than all other groups (Table 1). Orange jasmine → orange jasmine psyllids developed the slowest (Fig. 2B; Table 1). When ‘Valencia’ psyllids were transferred to orange jasmine, the development time increased, but was statistically similar to the orange jasmine → orange jasmine group (Fig. 2B; Table 2). However, when orange jasmine psyllids were transferred to ‘Valencia’ orange, the development time decreased, but remained slower than ‘Valencia’ → ‘Valencia’ psyllids (Fig. 2B; Table 2).

**Effects of Maternal Host Preference on Adult Offspring Size**

To determine whether psyllids benefited from maternal host fidelity, morphological measurements were taken of five body structures of the emerging adult psyllids. MANOVA showed that there was a significant effect for treatment (host transfer) across all five dependent variables (Table 3). Post-hoc analysis (Tukey’s HSD) showed significant differences in almost all comparisons of the control to either the experimental group (Table 2) or ‘Valencia’ psyllids maintained on the natal host species (Table 1). ‘Valencia’ psyllids increased in size when moved to ‘Valencia’ orange plants, while ‘Valencia’ psyllids decreased in size when moved to orange jasmine, except for ‘Valencia’ psyllids that did not display decreased abdominal size when moved to orange jasmine. There was also a significant effect for sex (Table 3). Female psyllids were consistently larger than males in all measurements.

**Effect of Maternal Host Preference on Adult Offspring Behavior**

The amount of time taken to make a selection in a Y-maze olfactometer varied by treatment. Psyllids transferred to ‘Valencia’ orange plants took longer to make a selection than those transferred to orange jasmine plants, regardless of the natal host (Fig. 4A). Orange jasmine reared psyllids reached the target 33% faster than those psyllids transferred to ‘Valencia’ orange (Wilcoxon sum-rank test: \( W = 439.5, P = 0.01 \)). Similarly, ‘Valencia’ orange psyllids transferred to orange jasmine reached the target 40% faster than those maintained on ‘Valencia’ orange plants (Wilcoxon sum-rank test: \( W = 600.5, P = 0.055 \)).

**Effect of Maternal Host Preference on Nymph Survival**

Although the mortality was low for all host transfer treatments, those maintained on their natal host species’ experienced the lowest mortality (Fig. 4B). Psyllids transferred from orange jasmine to ‘Valencia’ orange and those transferred from ‘Valencia’ orange to orange jasmine experienced the greatest mortality. ‘Valencia’ psyllids transferred to orange jasmine showed significantly greater mortality than psyllids maintained on ‘Valencia’ plants (\( \chi^2 = 4.35, P = 0.037 \)). However, there was no difference in mortality between orange jasmine reared psyllids maintained on orange jasmine and those transferred to ‘Valencia’ orange plants (\( \chi^2 = 1.58, P = 0.21 \)).

**Discussion**

Previous studies have indicated several forms of behavioral plasticity in *D. citri*. Males display learned attraction to female odor (Stockton et al. 2017a), and females appear to develop adaptive mate preferences based on experience with different mate phenotypes (Stockton et al. 2017b). Induced preference (Papaj and Prokopy 1989, Bernays and Weiss 1996) in female *D. citri* occurs in response to natal host experience, as well as short-term adult-feeding experience (Stockton et al. 2016). However, it was unknown whether such experience would coincide with differences in oviposition preference and offspring fitness. Our current study indicates that in addition to mate
We found that adult *D. citri* preferred to settle and oviposit on host plant genotypes with which they were exposed. Adult psyllids reared on 'Valencia' orange preferred to settle on 'Valencia' plants and psyllids reared on orange jasmine preferred to settle on orange jasmine plants. However, oviposition preference differed depending upon the host species with which the insects were experienced. While psyllids reared on 'Valencia' orange displayed a significant preference for 'Valencia' flush, orange jasmine psyllids were more likely to oviposit on both genotypes. Subsequently, we found that maternal preference may influence offspring fitness; we observed a direct effect of host fidelity on mortality. Host switching was associated with ~10–15% increase in mortality compared with nymphs maintained on the natal host genotype. Moreover, when 'Valencia' nymphs were moved to orange jasmine plants, those insects developed slower and decreased in size. Conversely, orange jasmine nymphs developed faster and increased in size if moved from orange jasmine to 'Valencia' orange. However, forced movement between hosts did not appear to affect psyllid activity level once they had completed adult development. Rather, our measure of activity level showed a decline in energetics associated with the host plant species rather than an effect of natal host experience. It is possible that the olfactory stimulant (β-caryophyllene) used in this assay was more attractive, and therefore more motivating, for psyllids recently exposed to orange jasmine plants. Previous research has shown that short-term adult psyllid experience with specific host plant volatiles can increase the preference for those volatiles and override natal-derived preferences (Stockton et al. 2016).

In the common sulfur butterfly, *Colias philodice* Latreille, host switching during late larval development is associated with decreased feeding, digestion, and larval weight, due to difficulty acclimating to a novel host (Karowe 1989). Similarly, the two-spotted spider mite (*Tetranychus urticae* Koch) can be reared on either bean or tomato plants, but display differential preference and performance depending on the host species with which they are acclimated (Agrawal et al. 2002). Tomato plants are more toxic than bean plants, and as a result, mites reared on tomato display up-regulated p450 enzymes (Agrawal et al. 2002). This increased detoxification ability allows mites reared on tomato to reproduce easily on both tomato and bean plants; whereas, mites reared on bean plants show decreased fecundity on tomato. We suggest that a similar effect may occur in *D. citri*.

Analysis of the volatile profiles has shown that relative to orange jasmine and curry leaf plants, 'Valencia' orange emits lower concentrations of defensive sesquiterpenes, particularly β-caryophyllene, germacrene-D, and β-cubebene (Robbins et al. 2012). In addition, psyllids reared on orange jasmine exhibit upregulated cytochrome p450 monooxygenase and glutathione s-transferase activity, which are both detoxification enzymes that allow psyllids to process defensive chemicals allelochemicals that would otherwise reduce herbivory (Liu et al. 2015). Insecticide resistant psyllid populations have been found with similar upregulated enzymes (Tiwari et al. 2013). Although the effect of host plant experience on insecticide susceptibility remains to be investigated, it is possible that insecticide

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**Table 1.** Diaphorina citri emergence patterns by treatment showing the total number of psyllids completing development (cumulative emergence = n), mean (x) development time, median (M) development time, and interquartile range (IQR) of the number of days required to complete development for each host transfer treatment

<table>
<thead>
<tr>
<th>Natal host</th>
<th>Transfer host</th>
<th>n</th>
<th>x</th>
<th>M</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Valencia' Orange</td>
<td>'Valencia' Orange</td>
<td>216</td>
<td>9.84</td>
<td>10</td>
<td>11–9</td>
</tr>
<tr>
<td>'Valencia' Orange</td>
<td>Orange Jasmine</td>
<td>161</td>
<td>11.42</td>
<td>12</td>
<td>13–10</td>
</tr>
<tr>
<td>Orange Jasmine</td>
<td>Orange Jasmine</td>
<td>210</td>
<td>11.73</td>
<td>11</td>
<td>13–10</td>
</tr>
<tr>
<td>Orange Jasmine</td>
<td>'Valencia' Orange</td>
<td>248</td>
<td>10.92</td>
<td>11</td>
<td>12–10</td>
</tr>
</tbody>
</table>

*Host species denoted as 'Valencia' (Val) and orange jasmine (OJ). Statistically significant comparisons are highlighted in bold.*

**Table 2.** Wilcoxon sum-rank test comparisons of emergence time for each pair of host transfer treatments (natal-host–transfer-host)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>W</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val → Val</td>
<td>Val → OJ</td>
<td>26,510</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Val → Val</td>
<td>OJ → Val</td>
<td>37,659</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Val → Val</td>
<td>OJ → OJ</td>
<td>33,400</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OJ → OJ</td>
<td>OJ → Val</td>
<td>29,444</td>
<td>0.014</td>
</tr>
<tr>
<td>OJ → OJ</td>
<td>Val → OJ</td>
<td>16,709</td>
<td>0.847</td>
</tr>
<tr>
<td>OJ → Val</td>
<td>Val → Val</td>
<td>16,324</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 3.** MANOVA comparisons of five adult psyllid body characteristics by treatment and sex

<table>
<thead>
<tr>
<th>Body characteristic</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>162.02</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>143.74</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Sex: Treatment</td>
<td>3</td>
<td>3.03</td>
<td>0.03*</td>
</tr>
<tr>
<td>Residuals</td>
<td>742</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femur</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>1.21</td>
<td>0.27</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>54.04</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Sex: Treatment</td>
<td>3</td>
<td>1.30</td>
<td>0.27</td>
</tr>
<tr>
<td>Residuals</td>
<td>742</td>
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<td></td>
</tr>
<tr>
<td>Head</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>53.90</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>54.31</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Sex: Treatment</td>
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<td>0.27</td>
<td>0.85</td>
</tr>
<tr>
<td>Residuals</td>
<td>742</td>
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<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>128.89</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>18.15</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Sex: Treatment</td>
<td>3</td>
<td>0.27</td>
<td>0.85</td>
</tr>
<tr>
<td>Residuals</td>
<td>742</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>145.83</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>32.01</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Sex: Treatment</td>
<td>3</td>
<td>0.54</td>
<td>0.65</td>
</tr>
<tr>
<td>Residuals</td>
<td>742</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Asterisks indicate statistically significant differences: *0.05; **<0.01; ***<0.001; ****<0.00001.

choice, oviposition preference may be influenced by early experience with certain host plant genotypes. In addition, we found fitness effects associated with learned maternal host choice, indicating host fidelity benefits in this species. This is consistent with previous studies, which have identified differences in *D. citri* development and body size depending on host plant genotype (García-Pérez et al. 2013, Paris et al. 2016), and suggests phenotypic variations may be associated with host plant genotype experience.
resistance is greater in psyllids reared on *Murraya* spp. This “pre-adaptation hypothesis” supposes that there are similar pathways involved in detoxifying both plant-produced allelochemicals and insecticides (Dermauw et al. 2013).

An alternative hypothesis is that poor performance was related to the physical structure of the host plants, such that ‘Valencia’ psyllids had greater difficulty feeding on orange jasmine plants. Physical barriers within a plant are a well-known defensive mechanism in plants. In citrus, the first and second instars of *D. citri* are restricted to feeding on the meristem and those leaves that have not opened (Tsai and Liu 2000). The stylet length of these early instars may be too short to reach phloem tissues in older leaves (Ammar et al. 2013). While adult styles are longer, adults feeding on older leaves have to find a path through or around a fibrous ring that protects the...

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**Fig. 3.** Morphometric comparisons of five adult psyllid body characteristics, wing length (A), femur length (B), head width (C), abdominal length (D), and body length (E) within each host-transfer treatment. Asterisks indicate significant results from post-hoc multiple comparisons of structure size (mean ±SEM) among psyllids initially reared on orange jasmine or ‘Valencia’ orange (x-axes) and transferred to ‘Valencia’ orange (gray bars) or orange jasmine (white bars) using t-tests. NS = non-significant, *<0.1, *<0.05, **< 0.01, ***< 0.001, ****< 0.0001.

**Fig. 4.** (A) Latency (s) to target selection for adult psyllids initially reared on orange jasmine or ‘Valencia’ orange plants (x-axis) and transferred to either orange jasmine (white bars) or ‘Valencia’ orange (gray bars). Asterisks indicate statistically significant differences in selection time in a Y-maze olfactometer, Wilcoxon sum-rank tests: *<0.05, **<0.01. (B) Nymph mortality following host transfer. The number of living (white bars) or dead psyllids (black bars) after 5 d was compared with chi-square tests. Different letters indicate statistically significant differences.

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vascular tissues (George et al. 2017). This ring increases in thickness and becomes more lignified with leaf age, and these changes influence psyllid feeding behavior (George et al. 2017). Psyllids reared on ‘Valencia’ may have initial difficulty learning to navigate the structural differences among different host genotypes. This may account for the greater mortality seen in our host transfer groups, indicating host fidelity benefits.

In addition to host volatiles and physical structure, there may also be differences in nutritional value per microliter of ingested phloem as well as differences in defensive chemistries among the host genotypes. The differences among sweet orange cultivars alone are highly significant as measured in sugar content, pH, amino acids, fatty acids, and organic acids (Killiny 2017). While the constituency of orange jasmine phloem was not studied, the significant differences present between the different cultivars of C. sinensis suggest that the differences between species could be even greater. In addition to nutritional differences, there are also constitutive and induced defensive differences. One group of defensive compounds is the tryptamines (Thomas et al. 1998, Gill et al. 2003) and these compounds or their derivatives are present in different levels in different citrus species (Servillo et al. 2017). One induced defense is chlorophyllid (Killiny and Nehela 2017) and it is possible that orange jasmine may have differential levels of induced chlorophyllide relative to sweet orange.

By selecting the host plant species on which they themselves have successfully developed, female D. citri may avoid the risks (chemical and structural defenses; nutritional differences) associated with unknown species. We propose that in some species, maternal host preference is guided by an interplay among three key players: 1) genetic preference; 2) learned preference; and 3) varying levels of adaptedness to certain host plants depending on the natal environment (Beltman and Metz 2005). Although females may have innate feeding preferences, those preferences may be modified by the experience to encourage alternative resource selection. In addition, the natal environment shapes maternal and offspring adaptedness to certain host species, further influencing future host selection. Due to incomplete natal host controls used in previous studies, it is unclear to what extent genetic preferences influence D. citri beyond general restriction to host plants in the family Rutaceae. It is possible that genetic preference in D. citri is limited to the selection of plants in the family Rutaceae, while selection within the family, particularly among plants within the genera Citrus and Murraya, is more experimental. However, future studies may clarify how this three-way interaction shapes psyllid preferences such that, for this disease vector species, more reliable movement and transmission models can be developed to deter the spread of HLB.

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