Exposure to Guava Affects Citrus Olfactory Cues and Attractiveness to *Diaphorina citri* (Hemiptera: Psyllidae)

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**Abstract**

Intercropping can reduce agricultural pest incidence, and represents an important sustainable alternative to conventional pest control methods. Understanding the ecological mechanisms for intercropping could help optimize its use, particularly in tropical systems which present a large number of intercropping possibilities. Citrus is threatened worldwide by greening disease (huanglongbing, HLB) vectored by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Control of HLB and citrus psyllid can be partially achieved through intercropping with guava, *Psidium guajava* L., but the mechanisms remain unclear. We tested the hypothesis that guava olfactory cues affect psyllid behavior by altering the attractiveness of citrus through plant–plant interactions. In choice and no-choice cage experiments, psyllid settlement was reduced on citrus shoots that had been exposed to guava shoot odors for at least 2 h. In Y-tube olfactometer experiments, psyllids oriented to odors of unexposed, compared with guava-exposed, citrus shoots. These behavioral results indicate that a mechanism for the success of guava intercropping for sustainable, ecological disease management may be the indirect effect of guava on citrus attractiveness.

**Key words:** plant–plant interaction, olfactory cue, *Diaphorina citri*, plant–insect interaction, *Psidium guajava*
guava and citrus, the protective effect against psyllids appears to function year-round (Rouseff et al. 2008), suggesting it may be mediated by leaf volatiles, rather than by the volatiles of seasonal fruit.

The volatile-mediated benefits of intercropping could result from two hypothetical mechanisms. First, guava volatiles could be directly repellent to psyllids, and thereby limit pathogen spread (direct repellence hypothesis). In support of this hypothesis, guava leaf volatiles inhibited attraction of psyllids to normally attractive citrus volatiles (Onagbola et al. 2011) in Y-tube olfactometer tests. Moreover, psyllid settlement on citrus was significantly reduced in the presence of guava leaves in laboratory cage trials (Zaka et al. 2010). Mechanically wounded guava produces toxic and repellent dimethyl disulfides, while citrus does not, suggesting these, or other guava compounds, may be responsible for the observed deterrence (Rouseff 2008). These results are consistent with other studies showing that specific volatiles of nonhost plants can disrupt host selection by insects (Huber and Borden 2001, Hassanalil et al. 2008).

Second, guava volatiles could be detected by citrus plants, leading to changes in the characteristics of citrus that mediate host selection. This indirect, or plant–plant communication, hypothesis was proposed by Baldwin and Schulz (1983), in their experiments with poplar (Populus × euramerica) and sugar maple (Acer saccharum Marsh), which showed that aerial cues released by mechanical damage to the plant leaves could cause biochemical changes in conspecific undamaged neighbors, and which reduced herbivore performance. These results have since been supported in other systems by several studies demonstrating that volatile based, plant–plant communication can cause associative resistance (Karban 2001, Barbosa et al. 2009, Jacet et al. 2011). For example, exposure to the volatiles emitted from thistle, Cirsium vulgare, reduced aphid acceptance of barley, Hordeum vulgare cv. Kara (Glinwood et al. 2004). Conversely, few studies have tested the effects of exposure to volatiles of undamaged neighbors, a critical context from a practical perspective (Ninkovic et al. 2013). Interplanting in Kenyan cropping systems has been successful in controlling insect herbivores implicating push–pull mechanism (Cook et al. 2007, Khan et al. 2009), and the success of the approach is attributed to the release of volatiles from companion plants (Poveda and Kessler 2012). An understanding of plant–plant interactions and associational resistance in intercropping systems would greatly facilitate the practical implementation of this approach, and yet few studies have examined this mechanism.

To gain a better understanding of the ecological mechanisms of guava–citrus intercropping, we tested the plant–plant interaction hypothesis in laboratory studies, investigating how exposure to putative guava volatiles altered Asian citrus psyllid attraction to citrus olfactory cues and subsequent host acceptance.

Materials and Methods
Insect and Plant Material
Adult D. citri were collected from 4-yr-old sweet orange (Citrus × aurantium L.) trees in the botanical garden (23° 15’ N, 113° 35’ E, 20 m altitude) of South China Agricultural University, Guangzhou, Guangdong, P. R. China, using a mechanical aspirator each morning and held in small plastic cups for use later in the day. Insects were sexed by an abdominal dimorphism, in which the tip of the male abdomen is bent upward and the female abdomen stays there for at least 1 min. Pooled responses of 10 individual psyllids comprised a single replicate, and 15 replicates were conducted in the laboratory over three days at 27 ± 2°C and light intensity of a 282 mW/cm². Between replicates, positions of the odor sources were exchanged to avoid directional bias, and the Y-tube was washed and dried.

Statistical Analysis
In the no-choice experiment, we used repeated-measures analysis of variance (ANOVA) to test the hypothesis that settlement of psyllids differed among the five exposure treatments, with treatment as a fixed effect, and time as the repeated measure. Number of psyllids
was averaged (pooled) across plants within a cage (the experimental unit) for this statistical analysis; however, data are graphically presented as the plant-wise means and standard errors due to the low cage replication at each time point (Fig. 1A). Treatment was modeled as a categorical variable; modeling as a continuous variable gave similar results. Data were log transformed to improve normality of the residuals and homoscedasticity. In the choice experiment, proportions of psyllids on each plant were arcsin-square root transformed and analyzed by repeated-measures ANOVA as for the no-choice experiment (Sokal and Rohlf 1995), followed by a Tukey’s test comparing the five treatments (averaged across the four assessment times). Repeated-measures analyses were run in the multivariate modeling platform of JMP v. 11.0, in a restricted maximum likelihood framework. For the Y-tube experiment, proportions of responders were arcsin-square root transformed and analyzed by ANOVA (general linear model) with treatment as a fixed effect (Sokal and Rohlf 1995). We note that for experiments involving proportions of responders, use of linear models (ANOVA) was preferred over \( \chi^2 \) tests, as ANOVA on transformed proportions considers variation among replicates, where available (as here). However, \( \chi^2 \) tests yielded qualitatively identical results. All analyses used JMP v11.0 (SAS Institute, 2012).

Results

Effect of Guava Exposure on Citrus Attractiveness

In the no-choice experiment, guava exposure significantly reduced the number of psyllids settling on citrus shoots (Treatment: \( F_{4, 5} = 5.510, P = 0.044 \); Fig. 1A). This effect was also significant when considering the linear relationship of settlement with exposure time, i.e., considering exposure as a continuous variable (\( F_{1, 8} = 22.67, P = 0.0014 \)). While this pattern of settlement appeared to attenuate at the later assessment times (e.g., 24 h after introducing psyllids to the cage, Fig. 1), the effects of assessment time were not statistically significant (Time: \( F_{3, 3} = 2.274, P = 0.2586 \); Treatment \( \times \) Time: \( \text{approx} F_{12, 8.2} = 1.387, P = 0.3256 \)). Relative to the unexposed control shoots, guava exposure for 30, 60, 120, and 180 min reduced settlement of psyllids by 14, 7, 47, and 46%, respectively (average of four exposure times).

In the choice experiment, psyllid settlement was proportionately greater on control shoots, and declined with increasing guava-exposure time (Fig. 1B; Treatment: \( F_{4, 35} = 23.459, P < 0.0001 \)). This effect appeared to be independent of the time of assessment (Time: \( F_{3, 33} = 0.2207, P = 0.8813 \); Treatment \( \times \) Time: \( \text{approx} F_{12, 87.6} = 0.4986, P = 0.9103 \)). A Tukey’s multiple comparison procedure on the transformed data (averaged across assessment times) indicated
that, relative to controls, there was no effect of 30-min guava exposure, but significant reductions in the average proportion alighting on citrus that had been exposed to guava for 60, 120, and 180 min. Overall alightment rates per cage were higher in the choice experiment (Fig. 1B Inset) than in the no-choice experiment (Fig. 1A).

**Response of Psyllids to Odor of Guava-Exposed Citrus**

In the Y-tube olfactometer, 61.3% of psyllids oriented to odors of control citrus, over citrus that had been exposed to guava for 3 h (38.7%; \( F_{1, 28} = 5.21, P < 0.0001; \) Fig. 2).

**Discussion**

Our results show that when citrus is exposed to guava, there is reduced settlement of adult Asian citrus psyllid, indicating less acceptability of guava volatile-exposed citrus shoots to the herbivores. These results support the indirect plant–plant interaction hypothesis for the effects of guava intercropping on psyllids. Behavior of herbivores and their natural enemies can be affected by the exposure of plants to volatiles of another plant of same species or different species. For example, in certain undamaged barley cultivars, exposure to volatiles of conspecific (Glinwood et al. 2009, Ninkovic and Ahman 2009) or heterospecific neighbors (Glinwood et al. 2004) reduces host acceptability to aphids and increases attractiveness to predatory ladybirds (Ninkovic and Pettersson 2003). The relation across multitrophic levels influenced by chemical interactions among neighboring plants is allelobiosis (Ninkovic et al. 2006), and our results are consistent with an allelobiosis interpretation of guava–citrus–psyllid interactions.

Our Y-tube experiment confirms that the indirect mechanism observed in the settlement assays is mediated at least in part by olfactory responses of psyllids to altered citrus shoot odor. Response of Asian citrus psyllid to citrus odors was also affected by the volatiles of *Allium* spp. in T-olfactometer tests (Mann et al. 2011). These results complement prior olfactometer experiments showing that *D. citri* were directly repelled by odors from mechanically wounded guava leaves (Onagbola et al. 2011), and that odor of fresh guava leaf volatiles, alone or in combination with citrus leaf volatiles, were repellent to psyllid adults in a dose-dependent manner (Zaka et al. 2010). Thus, both direct repellence and indirect plant–plant interaction mechanisms may operate in this system. However, determining the relative importance of the two mechanisms in a field setting remains an important goal for future research.

Avoidance of guava-exposed citrus by psyllids may reflect an avoidance of plants of reduced quality, consistent with several studies of plant–plant volatile interaction in which herbivory-induced volatiles increased defense activation in neighboring plants (Engelberth et al. 2004, Ton et al. 2007). Our study does not allow us to determine whether responses by psyllids reflect decreased attractiveness of citrus, or changes to repellent compounds. Prior studies have established a role of terpenoid volatiles in mediating host finding and acceptance by many insects, including Asian citrus psyllid (Sanchez 2009, Patt and Setamou 2010). Preliminary analysis of the volatile emissions of citrus shoots indicated that total monoterpenoid emissions show a significant threefold reduction after exposure to guava shoots (J. C. B., unpublished data); however, the role of specific volatile compounds in mediating the psyllid responses we report here remains to be verified.

Understanding plant–plant interactions is of interest from both an ecological perspective but also for the development of novel crop protection strategies that involve either engineering or selecting resistant crop plants or treating crops with allelopathic agents to make them less attractive to insects (Agelopoulos et al. 1999). The success of this approach depends in part on the target crop being sensitive to volatiles from an emitter intercrop that may or may not be attacked, and yet extremely few studies have examined crop responses to volatiles of undamaged neighbors. Consistent with the results of the present study, Ninkovic et al. (2013) reported that potato plants exposed to onion plants showed a fourfold increase in emission of two terpenoids, and a significant decrease in their attractiveness to winged aphids. The similarity of these results with the guava–citrus system strongly suggests that future studies of intercropping systems may identify additional cases of associational resistance based on plant–plant communication from undamaged neighbors.

Finally, interplanting of guava may be an option of protecting against psyllids in citrus groves. There are examples of compounds known to be allelopathic agents in plant–plant interactions that...
directly repel or deter herbivores, e.g., caffeine (Kim et al. 2006). Our study establishes an aerial allelopathic interaction between guava and citrus plant that can make the host less acceptable to psyllids, potentially by changing its volatile emissions. The present study also suggests that future research should focus on understanding the behavioral activity of volatiles in the naturally mixed vegetation of citrus and guava; such studies could indicate whether it is possible to optimize guava planting to take advantage of behaviorally active volatile compounds in integrated pest management of Asian citrus psyllids.

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References Cited


Aubert, B. 1987. Tryoza erytreae Del Guercio and Diaphorina citri Kuwayama (Homoptera: Psyllidae), the two vectors of citrus greening disease: Biological aspects and possible control strategies. Fruits 42: 149–162.


Sanchez, A. 2009. Importance of visual stimuli and host plant odor in host finding by the Asian citrus psyllid Diaphorina citri Kuwayama (Hemiptera: Psyllidae). MS thesis Texas A & M University-Kingsville, p. 112.


