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Repellency of selected *Psidium guajava* cultivars to the Asian citrus psyllid, *Diaphorina citri*

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ABSTRACT

Huanglongbing (HLB) is the most devastating disease of citrus worldwide. It is caused by bacteria of the genus '*Candidatus* Liberibacter' and transmitted by two psyllid species, the Asian citrus psyllid (ACP) *Diaphorina citri*, and the African citrus psyllid *Trioza erytreae*. Considerable research has been conducted toward developing and implementing HLB and ACP management strategies. With respect to ACP control, of interest is that reports indicate guava, *Psidium guajava*, can be repellent to ACP. We conducted research to further evaluate repellency of guava to ACP. In one set of experiments, guava oil from five Brazilian guava cultivars ('J3', 'Pedro Sato', 'Século XXI', 'Thailand' and 'Paluma') was extracted from leaves (immature and mature) by hydro-distillation in a Clevenger-type apparatus and evaluated for psyllid repellency. In a second set of experiments, repellency of guava leaves to ACP was investigated using leaves (immature and mature) from two guava cultivars in Florida, 'Pink' and 'Thai White'. In each set of experiments, repellency was evaluated by releasing ACP adults into a cage with two large vials, one containing a young flush shoot (= immature leaves) of *Murraya exotica* (a favored host plant of the psyllid, the flush of which is highly attractive to ACP) and one with *M. exotica* flush and the test material of interest (guava oil, immature guava leaf or mature guava leaf). The adults were free to move throughout the cage and into the vials, and the number of psyllids in each vial was counted after 24 h. The results showed that all guava materials tested had at least some repellency to ACP. Mature leaves tended to have a greater repellent effect than immature leaves. Each of the five oils exhibited repellency. A report in the literature suggested that sulfur compounds associated with guava may be responsible for ACP repellency. Interestingly, the five guava oil extracts we studied were repellent to ACP but none contained any sulfur compounds. Identification of the constituents responsible for repellency could lead to new ACP management tactics.

1. Introduction

Huanglongbing (HLB) is a devastating disease of citrus caused by bacteria in the genus '*Candidatus* Liberibacter' known to be transmitted by two psyllid species, the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama, and the African citrus psyllid *Trioza erytreae* (del Guercio) (Bové, 2006; Gottwald, 2010; Hall et al., 2013a). HLB attributed to '*Ca.* Liberibacter asiaticus' and vectored by ACP is considered to be Asian in origin and has spread to other citrus growing regions around the world including Brazil and the

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United States of America (USA). In addition, HLB attributed to '*Ca.* Liberibacter americanus' vectored by ACP was discovered in Brazil (Gottwald, 2010). Citrus trees infected by HLB pathogens become unproductive with thinning canopies, juice from the fruit of infected trees develop off-flavors, the disease promotes premature fruit drop, and infected trees may eventually die. The citrus industries in Brazil and USA and other areas where HLB has spread have scrambled to find solutions with little success. In Brazil, citrus growers attempt to manage HLB using a three-tiered approach: only plant new trees that are free of the disease, establish and maintain aggressive insecticide programs for psyllid control, and aggressively find and remove infected trees to reduce inoculum loads. In the USA where HLB is currently jeopardizing the Florida citrus industry, citrus growers initially followed the same three-tiered program, but the high cost of identifying and removing infected trees and reluctance to remove infected trees that were still productive led to most growers abandoning the tree-removal component of this HLB management program (Hall et al., 2013a). Furthermore, aside from the fact that intensive insecticide programs are not sustainable, even the most intensive insecticide programs against ACP have provided little protection against the introduction and spread of HLB in new plantings (Hall et al., 2013b). Solutions to HLB remain desperately needed.

The pursuit of improved management tactics for ACP includes research in the area of chemical ecology searching for attractants and repellents. An attractant could be used for ACP surveillance and possibly for mating disruption. A repellent could be used to drive ACP away from citrus. With respect to attractants, Wenninger et al. (2008) reported behavioral evidence of an ACP sex pheromone, although to-date none have been identified. With respect to ACP repellents, Beattie et al. (2006) reported that, in Vietnam, citrus intercropped with guava (*Psidium guajava* L., plant family Myrtaceae) had a lower incidence of HLB compared to citrus planted alone, possibly due to the presence of volatiles associated with guava that repelled ACP. Since then, a number of research efforts have been made on repellency of guava and other plant species to ACP including Chen et al. (2006); Hall et al. (2008); Onagbola et al. (2011); Rouseff et al. (2008); Gottwald et al. (2010); Zaka et al. (2010); Mann et al. (2012); and Robbins et al. (2012). Gottwald et al. (2014) reported that the Vietnamese guava effect could not be verified in Florida citrus due to problems with nematodes and sensitivity of guava to cold weather. Ultimately, even if guava could be grown in an area, it would likely promote problems with fruit flies in citrus. As an alternative to intercropping guava and citrus, if guava volatiles with repellency to ACP could be identified, it might be possible to use these against ACP without requiring that guava be grown with citrus.

The goal of research presented here was to further assess repellency of guava to ACP.

2. Materials and methods

Two experiments were conducted to assess repellency of guava to ACP. In one experiment, ACP attraction and settling behavior were studied on young flush shoots of orange jasmine in the presence or absence of oil extracts from one of five different guava cultivars. Orange jasmine [*Murraya exotica* L. (= *Murraya paniculata* auct. non.)] is a favored host plant of ACP (Hall and Rohrig, 2015). ACP reproduction is dependent on young flush shoots (Husain and Nath, 1927), which are immature or young leaves as described by Hall and Albrigo (2007). In the second experiment, ACP attraction and settling behavior were studied using orange jasmine flush shoots in the presence or absence of guava leaves.

2.1. Repellency of guava oils to adult ACP

Essential oils from fresh immature and mature leaves of five guava cultivars were studied, three of red pulp commercially known as 'Paluma', 'Pedro Sato', and 'Século XXI'; one of white pulp identified as 'Thailand'; and one new selection of red pulp identified as 'J3' and considered to have some repellency to the guava psyllid *Triozoida limbata* (Hemiptera: Triozidae). Young and mature leaves from these cultivars were collected in September 2013 from six-month-old, potted plants propagated by rooting of semi-hardwood cuttings. The essential oils were obtained by hydrodistillation for 3 h of 50 g leaf material in 500 mL water using a Clevenger-type apparatus, adapted according to the method described in British Pharmacopoeia (1980). The volatile compounds were extracted from the distillation water with dichloromethane, dried over anhydrous sodium sulphate and carefully concentrated under N_2 to a final volume of <0.5 mL, and then analyzed by gas chromatography/mass spectrometry (GC/MS) using a Shimadzu QP5050A system equipped with J&W Scientific DB-5 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness); column temperatures were programmed from 60 °C for 3 min, raised to 150 °C at 8 °C/min, isotherm of 5 min, raised to 280 °C at 12 °C/min and isotherm of 5 min. Injector and detector temperatures were 250 °C and 280 °C, respectively. Helium was used as carrier gas, with flow rate of 1.5 mL/min, split mode. Injection volume was 1.0 μL solution in dichloromethane. The MS were taken at 70 eV. Scanning speed was 0.5 scan/sec from *m*/*z* 50 to 500. The retention indices were obtained by injecting the C_{10}—C_{29} linear hydrocarbon mixture. The percent composition of each component was determined from the area of the component divided by the total area of all components isolated under these conditions. The volatile components were analyzed by GC/MS, and identification was made on the basis of comparison of retention indices as well as by computerized matching of the acquired mass spectra with those stored in the National Institute of Standards and Technology's mass spectral library of the GC/MS data system and other published mass spectra. To test the guava oils for repellency to ACP, 20 μg of each guava oil was mixed into 10 mL of SPLAT™ (ISCA Technologies, Inc., Riverside, CA), an emulsified wax substrate for slow release of insect semiochemicals (Lapointe et al., 2011). A cotton wick was treated with 1.0 g of SPLAT containing guava oil. Assays were then conducted in which ACP attraction to young flush shoots of orange jasmine with and without a cotton wick containing guava oil was assessed as described below.

ACP attraction to flush in the presence or absence of guava oil was assessed using a behavioral assay described by Hall et al. (2015). Two large vials each containing young flush shoots of orange jasmine (a combined average weight of flush per vial of 0.27 ± 0.05 g) were placed into an assay cage (described below). One vial also received a cotton wick treated with one of the guava oils in SPLAT. In addition to the five guava oil treatments, a sixth treatment was included in which one vial of orange jasmine flush received a cotton wick treated with SPLAT not containing any oil. The assay vials were 25 dram plastic tubes measuring 39 × 85 mm I.D. (diameter × height) (#8925, BioQuip Products, Inc., Gardenia, CA) with white snap-on plastic lids. We used a cork borer to cut a 6 mm diameter hole through each vial's lid. Flush shoots for the assay were excised from potted plants in a greenhouse; the cut end of each flush shoot was slipped into a 1.5 mL centrifuge tube containing tap water and secured to the tube with Parafilm M®laboratory film (American National Can, Chicago, IL). Two tubes each with flush were placed into each assay vial, and each tube with flush was held in an upright position in the vial by a plastic support. Each plastic support was the bottom half of one of the small centrifuge tubes, cut in half with the top half discarded and the