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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

Table 1
Volatile composition of young and mature leaves of varieties of *Psidium guajava* L.

Constituents	RI[a] (calc.)	RI[b] (lit.)	Peak area (%)				
			'J3'	'Thailand'	'Paluma'	'Pedro sato'	'Sec XXI'
α-Pinene	940	939	7.20	–	–	–	–
Benzaldehyde	961	960	–	0.50	–	–	0.59
Limonene	1030	1029	22.59	0.53	–	–	0.22
1,8-Cineole	1035	1031	–	0.61	–	1.98	2.85
(Z)-β-Ocimene	1040	1037	3.36	0.17	–	0.94	0.89
δ-Elemene	1339	1038	–	0.10	–	–	–
α-Cubebene	1350	1351	–	0.18	–	–	–
α-Copaene	1379	1377	1.13	4.17	2.48	5.16	0.25
Isocaryophyllene	1406	1408	–	0.21	–	–	0.06
β-Caryophyllene	1422	1419	20.54	33.14	45.56	30.10	21.93
α-Guaiene	1438	1440	–	3.17	–	–	–
Aromadendrene	1443	1441	1.45	–	4.28	–	–
α-humulene	1458	1455	2.70	5.74	6.17	6.39	3.86
Allo-aromadrendene	1460	1460	–	1.47	–	–	–
9-epi-(E)-Caryophyllene	1467	1466	0.75	–	–	–	–
Drima-7,9-diene	1477	1473	–	–	–	0.35	0.36
β-Chamigrene	1477	1478	–	–	–	–	1.81
γ-Muurolene	1477	1480	–	0.47	–	–	–
γ-Gurjunene	1481	1480	0.62	–	–	1.73	–
Germacrene D	1482	1485	–	–	1.16	–	–
β-selinene	1490	1490	2.34	0.64	12.87	18.92	15.64
γ-amorphene	1493	1496	–	0.20	–	–	–
α-Selinene	1498	1498	2.57	0.71	13.19	15.02	12.87
Ni	1501	–	–	0.85	–	–	–
(E,E)-α-farnesene	1506	1506	2.45	–	–	–	–
β-Bisabolene	1507	1506	–	0.43	–	–	–
Ni	1512	–	1.93	–	–	–	–
γ-Cadinene	1513	1514	1.19	0.31	–	–	–
7-epi-α-Selinene	1515	1522	–	–	–	–	0.43
Ni	1519	–	–	–	–	0.35	
δ-Cadinene	1525	1523	2.31	–	1.55	1.54	0.43
trans-Cadina-1,4-diene	1530	1535	–	1.61	–	–	–
(E)-γ-Bisabolene	1535	1531	0.21	–	–	–	–
Ni	1539	–	–	–	–	0.36	–
Ni	1551	–	–	–	–	–	0.61
Ni	1559	–	–	1.21	–	–	–
(E)-Nerolidol	1563	1563	18.50	17.03	–	–	4.30
Caryophyllenyl alcohol	1571	1572	–	–	0.64	–	0.53
Spathulenol	1581	1578	–	2.43	–	–	–
Caryophyllene oxide	1587	1583	–	4.01	–	1.41	5.08
Globulol	1587	1585	–	5.84	–	–	–
Ni	1592	–	2.20	–	–	–	–
Viridiflorol	1596	1593	–	1.26	5.44	1.90	–
Ni	1606	–	–	2.68	–	–	–
Humulene epoxide II	1611	1608	–	–	–	–	0.50
Ni	1615	–	–	–	–	–	1.59
Ni	1618	–	0.29	–	–	–	0.46
			'J3'	'Thailand'	'Paluma'	'Pedro sato'	'Sec XXI'
Ni	1621	–	–	–	–	1.27	–
Ni	1623	–	–	–	0.41	–	0.35
1-epi-cubenol	1628	1629	0.75	2.11	0.66	1.25	–
Ni	1629	–	–	–	–	–	1.25
cis-Cadin-4-en-7-ol	1633	1637	–	–	–	0.72	–
Caryophylla-4,8-dien-5β-ol	1638	1641	–	–	–	–	6.82
α-Muurolol	1641	1646	–	1.95	–	–	–
Caryophylla-4,8-dien-5α-ol	1643	1641	–	–	–	0.72	1.45
Cubenol	1649	1647	–	1.29	–	0.40	–
Ni	1651	–	–	–	–	0.39	–
Selin-11-en-4α-ol	1660	1660	2.35	–	5.59	9.10	13.61
Ni	1667	–	–	–	–	–	0.70
Ni	1683	–	–	–	–	–	0.56
Total			97.43	95.63	100	100	99.41

Ni = unidentified compounds; - = not observed.
[a] RI (calc.) = retention indices on DB-5 column.
[b] RI (lit.) = retention indices according to the literature.

bottom half hot-glued in an upright position to the inside bottom wall of the vial – a centrifuge tube fitted with a flush shoot in water was slipped down into each plastic support. After the two tubes with flush were placed into a vial, the lid was snapped into place and the exterior clear wall of each vial was covered with white paper to conceal the contents of the vial, thus preventing visual attraction to flush but allowing odors associated with flush to attract ACP into a vial through the lid's hole.

Table 2
Percentage of each chemical group present in guava oil from each of five Brazilian guava cultivars.

Chemical group	Guava cultivar				
	'J3'	'Pedro Sato'	'Século XXI'	'Thailand'	'Paluma'
Monoterpenes	33.2	0.9	1.1	0.7	0.0
Oxygenated Monoterpenes	0.0	2.0	2.9	0.6	0.0
Sesquiterpenes	38.3	79.2	57.6	52.6	87.3
Oxygenated Sesquiterpenes	21.6	15.5	32.3	35.9	12.3
Benzenoids and other compounds	4.4	2.4	5.5	5.9	0.4
Totals	97.4	100.0	99.4	95.6	100.0

Table 3
Location of adult Asian citrus psyllids 24 h after being introduced into a cage with free choice to move into one of two assay vials, one with Murraya exotica flush or one with M. exotica flush and guava oil. Guava oil was obtained from five different cultivars. Means followed by same letter (lowercase vertically or uppercase horizontally) are not significantly different (Tukey's test, $P < 0.05$), analyses on arcsine-transformed percentages, raw mean percentages presented. (For *F* values, ns = not significant, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$; SMD: significance minimum difference; VC: variation coefficient).

Guava cultivar oil treatment	Mean percentage of psyllids at the indicated location					
	Not in a vial	In the vial with only *Murraya* flush	In the vial with *Murraya* flush + oil	$F_{2,18}$ value	SMD	VC %
'J3'	21 a B	64 a A	15 b B	44.84**	9.19	23.79
'Pedro Sato'	28 a B	49 bc A	23 b B	25.02**	5.98	15.02
'Século XXI'	28 a B	52 b A	20 b B	25.49**	7.26	18.32
'Thailand'	26 a B	50 b A	24 b B	22.29**	6.74	16.96
'Paluma'	25 a B	54 ab A	21 b B	22.78**	8.37	21.14
Control (no oil)	24 a B	37 c A	39 a A	3.94*	9.78	24.58
$F_{2,54}$ value	1.34 ns	9.70**	9.63**			
SMD	8.66	6.92	7.24			
VC %	22.04	11.49	19.21			

Table 4
Location of adult *Diaphorina citri* 24 h after being introduced into a cage with free choice to move into one of two assay vials, one with *Murraya exotica* flush or one with either *M. exotica* flush by itself (cage 1 treatment), *M. exotica* flush with 'Pink' guava flush (cage 2 treatment), or *M. exotica* flush with a mature 'Pink' guava leaf (cage 3 treatment). Means followed by same letter ((lowercase vertically or uppercase horizontally) are not significantly different (Tukey's test, $P < 0.05$), analyses on transformed percentages, raw mean percentages presented. (For *F* values, ns = not significant, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$; SMD: significance minimum difference; VC: variation coefficient).

Treatment	Mean percentage of psyllids at the indicated location					
	Not in a vial	In the vial with only *Murraya* flush	In the vial with only *Murraya* flush, *Murraya* flush with guava flush, or *Murraya* flush with a mature guava leaf	$F_{2,30}$ value	SMD	VC %
Cage 1 (no guava)	21 a B	41 a A	38 a A	6.02**	9.86	32.86
Cage 2 (guava flush)	32 a A	41 a A	27 ab A	1.80 ns	10.75	35.74
Cage 3 (mature guava leaf)	29 a B	47 a A	24 b B	6.75**	10.01	33.26
$F_{2,45}$ value	2.96 ns	0.72 ns	3.31*			
SMD	7.52	8.75	8.38			
VC %	28.50	25.18	30.24			

Table 5
Location of adult Asian citrus psyllids 24 h after being introduced into a cage with free choice to move into one of two assay vials, one with *Murraya* exotica flush or one with either *M. exotica* flush by itself (cage 1 treatment), *M. exotica* flush with 'Thai White' guava flush (cage 2 treatment), or *M. exotica* flush with a mature 'Thai White' guava leaf (cage 3 treatment). Means followed by same letter (lowercase vertically or uppercase horizontally) are not significantly different (Tukey's test, $P < 0.05$), analyses on transformed percentages, raw mean percentages presented. (For *F* values, ns = not significant, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$; SMD: significance minimum difference; VC: variation coefficient).

Treatment	Mean percentage of psyllids at the indicated location					
	Not in a vial	In the vial with only *Murraya* flush	In the vial with only *Murraya* flush, *Murraya* flush with guava flush, or *Murraya* flush with a mature guava leaf	$F_{2,30}$ value	SMD	VC %
Cage 1 (no guava)	25 a B	36 b AB	39 a A	6.14**	8.00	26.47
Cage 2 (guava flush)	14 b B	37 b A	50 a A	17.97**	10.23	34.55
Cage 3 (mature guava leaf)	26 a B	48 a A	25 b B	11.88**	7.38	24.38
$F_{2,45}$ value	6.49**	4.29*	15.19**			
SMD	7.05	7.03	6.79			
VC %	30.77	21.42	20.56			

The two assay vials containing shoots with or without guava oil were placed together onto the floor of a cubic cage ($30 \times 30 \times 30$ cm), with the vials positioned 10 cm from each other. The assay cage (Bugdorm-1 insect cage, DP1000, MegaView Science

Co., Ltd., Taichung, Taiwan) was screened (24 × 24 mesh/6.5 cm^2) on three sides with three solid sides made of a soft, thin, translucent material. A net sleeve through one of the solid sides allowed introduction and removal of the vials. The cage was placed into an environmental chamber set at 27 °C, 24 h daily illumination (humidity was not controlled but averaged 44%). Fifty adult ACP (~10 days old) were aspirated into a small glass vial which was placed onto the floor of the cage at the center of the cage between the two assay vials with flush. The adults were free to exit the small glass vial. The number and gender of adults in each assay vial, and the number and gender of adults found in the cage outside of the assay vials, were determined 24 h later. The data variable of interest was the percentage of ACP ending up in vials containing guava oil. Each guava oil treatment was studied individually one after another and all treatments were replicated 10 times. For each new replication of each treatment, the position of the assay vial with oil in the cage was reversed; in addition, there were three environmental chambers with the indicated settings, and treatments were systematically rotated among the three chambers.

2.2. Repellency of guava leaves to adult ACP

The same basic assay used to screen guava oils for repellency to ACP was used to screen guava leaves for repellency, with two assay vials containing leaf material in each of three cages. There were three cage treatments: 1) a cage with two vials each containing orange jasmine flush; 2) a cage with one vial containing orange jasmine flush and the second vial containing orange jasmine flush plus a young guava flush shoot; and 3) a cage with one vial containing orange jasmine flush and a second vial containing orange jasmine flush plus an intact mature guava leaf (the cut end of which was secured in a 1.5 mL centrifuge tube containing water). Two guava cultivars were studied, 'Pink' and 'Thai White'. 'Thai White' was a white guava cultivar commonly grown in South Vietnam - although similar in appearance to the Brazilian 'Thailand' cultivar, it was not known if these were genetically the same. For vials containing orange jasmine or guava flush shoots, there was an average of 0.27 ± 0.05 and 0.62 ± 0.09 g of flush per vial, respectively; in vials with mature guava leaves, there was an average of 1.80 ± 0.60 g of mature leaf tissue per vial. The assay was repeated 16 times for each cultivar, rotating the treatments among the three environmental chambers and switching the position of the vials in each cage. All replications for one cultivar were completed before starting those for the second cultivar.

2.3. Longevity and oviposition of adult ACP confined to guava

Longevity and oviposition activity by ACP on guava in a no-choice setting was investigated using potted plants placed into BugDorm-2 cages (#2120, 60 × 60 × 60 cm, MegaView Science Co., Ltd., Taichung, Taiwan). A group of 100 adult ACP (~10 days old) was placed into a cage with a 'Pink' guava plant, another group of 100 adults was placed into a cage with a 'Thai White' guava plant, and a third group of 100 adults was placed into a cage with *Citrus macrophylla* Wester ('Alemow'), a citrus plant known to be susceptible to ACP. In addition, a fourth group of 100 adults was placed into an empty cage. The cages were maintained in a greenhouse under ambient conditions and checked daily over 10 days to count numbers of dead ACP and to determine if oviposition occurred.

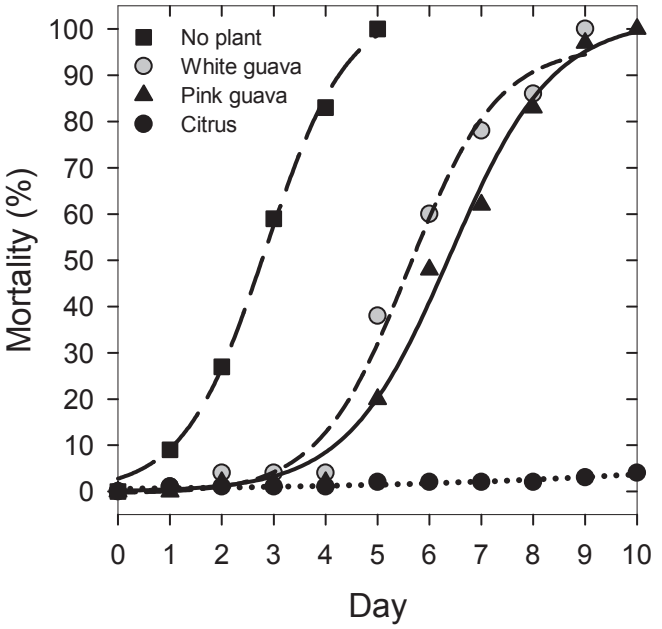

Fig. 1. Mortality of adult *Diaphorina citri* over a ten day period when they were caged without a plant compared to mortality of adults caged with one of the following plants: *Psidium guajava* 'Thai White', *P. guajava* 'Pink' or citrus. Fit of the logistic model to the different treatments was as follows. Alone: $r^2 = 0.99$, F = 1,509, P = <0.0001. 'Thai White': $r^2 = 0.99$, F = 489, P = <0.0001. 'Pink': $r^2 = 0.99$, F = 735, P = <0.0001. Citrus: $r^2 = 0.83$, F = 52, P < 0.0001. Refer to Table 6 for results of slope comparisons based on logistic regression.

2.4. Data analyses

Data from the studies with guava oil and guava leaves were subjected to analyses of variance (completely randomized design for comparisons among treatments or randomized block design for comparisons within treatments), and mean differences among treatments were investigated using Tukey's studentized range

Table 6
Location of adult Asian citrus psyllids 24 h after being introduced into a cage with free choice to move into one of two assay vials, one with *Murraya exotica* flush by itself and one with *M. exotica* flush plus guava. A young guava flush shoot was used in one set of assays and a mature guava leaf was used in a second set of assays. Two guava cultivars were studied, 'Pink' and 'Thai White'. Means followed by same letter (lowercase vertically or uppercase horizontally) are not significantly different (Tukey's test, P < 0.05), analyses on transformed percentages, raw mean percentages presented. (For *F* values, ns = not significant, * = significant at P = 0.05, and ** = significant at P = 0.01; SMD: significance minimum difference; VC: variation coefficient).

Vial contents	Mean percentage of psyllids at the indicated location						
	Guava flush		Guava mature leaf				
	'Pink'	'Thai white'	'Pink'	'Thai white'	$F_{1,60}$ value	SMD	VC %
Murraya flush alone	66 a B	71 a B	81 a AB	93 a A	8.10**	14.16	23.15
Murraya flush plus guava	34 b A	29 b A	19 b AB	7 b B	8.10**	14.16	61.63
$F_{3,15}$	5.06*	37.16**	7.92*	215.20**			
SMD	21.92	8.85	15.38	9.79			
VC %	64.88	27.22	45.42	30.12			

Table 7
Mortality curves for adult Asian citrus psyllid confined in cages with 'Thai White' guava, 'Pink' guava, or *Citrus macrophylla* plants or without plant material, comparison of slopes (β_1) from logistic-transformed curves of psyllid mortality. Refer to Fig. 1 for an overview of the curves.

Contents of cage	Regression slope (β_1)	β_1 standard error	Student's *t*-value			
			N	No plant material	'Thai white' guava	'Pink' guava
No plant material	5.323482	0.869062	11			
'Thai White' guava plant	3.722321	1.051161	9	−8.7928[a]		
'Pink' guava plant	2.910177	1.087584	8	−11.0438[a]	−22.2973[a]	
Citrus plant	0.56281	0.305311	5	8.4446[a]	4.2361[b]	3.0007[c]

[a] $P = <0.0001$. [b] $P = 0.002$. [c] $P = 0.015$.

(HSD) test. Percentage data were arcsine-transformed for these analyses ($arcsine\sqrt{x/100}$). ACP longevity data were logistic-transformed (Log[x/(1-x)]) and subjected to logistic regression to compare survival on guava to survival on citrus. All statistical tests were conducted at the 0.05 level of significance.

3. Results and discussion

Results of the chemical analyses on volatiles associated with oil extracts from the five guava cultivars are shown in Tables 1 and 2. β-caryophyllene was identified as a major constituent in oil from each of the five Brazilian guava cultivars, but in most other respects volatile constituents of the five oils differed substantially. Alquezar García et al. (2011) reported that the components β-caryophyllene and α-copaene have repellency to ACP. However, β-caryophyllene is common in orange jasmine (Raina et al., 2006; Lv et al., 2013), and ACP is highly attracted to orange jasmine. The guava oil 'J3' contained a high percentage of limonene, a common compound in *Citrus* species and reported to be an ACP attractant (Patt and Sétamou, 2010), and α-pinene which was not associated with oil from the other cultivars. Co-dominant compounds may be particularly important with regard to repellency to ACP. Sesquiterpenes were a major constituent of each of the five guava oils, especially in oil from 'Paluma', and oil from cultivar 'J3' was markedly different in that it was the only oil containing a large percentage of monoterpenes (Table 2).

Large percentages of ACP showed responses to odors in the guava oil assays, with from 72 to 79% of adults (mean of 75%) entering and settling in one of the two vials (Table 3). There was no preference between the two vials containing orange jasmine flush when no guava oil was present. However, significantly fewer adults entered and settled on flush when guava oil was present. This was true for all five guava cultivars, with from 2 to 4 (mean of 2.7) times more adults ending up in vials without guava oil. For the five guava oil treatments, an average of 54% of ACP chose the vial with only jasmine flush compared to 21% choosing the vial with both jasmine flush and guava oil, a reduction of 33%. Zaka et al. (2010) indicated a reduction of 52.7% in the number of psyllids on citrus leaves when guava foliage was present, and reported that immature and mature leaves showed equal repellent activity.

In the experiments with 'Pink' guava leaves, large percentages of ACP entered and settled in vials (from 68 to 79% and a mean of 73%) (Table 4). There were no significant differences in percentages of ACP ending up in the two vials when each contained orange jasmine flush. There were also no significant differences in percentages of ACP ending up in vials when one contained jasmine flush and one contained 'Pink' guava flush, although there tended to be fewer in the vial with guava flush. However, significantly greater percentages of ACP ended up in vials containing jasmine flush when the other vial contained a mature 'Pink' guava leaf, indicating a repellent effect associated with mature leaves.

From 73 to 87% (mean 78%) ACP chose and settled in vials during experiments with 'Thai White' guava leaves (Table 5). Similar to the results with 'Pink' guava leaves, there were no significant differences in percentages of ACP ending up in the two vials when each contained orange jasmine flush, and significantly greater percentages of ACP ended up in vials containing orange jasmine flush when the other vial contained a mature 'Thai White' guava leaf. However, unlike the experiments with 'Pink' guava leaves, adult ACP were significantly attracted into vials containing 'Thai White' flush, indicating that flush of this cultivar has an ACP attractant. These results differed from those of Zaka et al. (2010) who reported equal repellent activity when comparing immature and mature guava leaves placed beside a citrus shoot.

Analyses of combined data from the experiments with 'Pink' and 'Thai White' guava indicated that both immature and mature leaves of each cultivar were repellent, that mature leaves of 'Thai White' were significantly more repellent than immature leaves, and that repellency to ACP of mature leaves was similar between the two cultivars (Table 6). Gottwald et al. (2014) reported a significant reduction in field infestations of ACP in citrus intercropped with 'Pink' guava but not in citrus intercropped with 'Thai White' guava. Repellency to ACP of any guava cultivar could be positively or negatively affected by a number of abiotic and biotic factors.

Nearly 100% of adult ACP survived over a ten-day period when they were confined to citrus (Fig. 1), and large numbers of eggs were observed on this plant. When adults were confined in a cage without a host plant, 100% mortality occurred within five days; Hall and McCollum (2011) reported similar longevity when they held adults without a food host. Adult ACP confined to guava lived longer than adults held without a host plant; they were often observed in a feeding position on leaves, and the adults spent most of their time on the guava plants rather than on the floor or sides of the cages. However, 100% of the ACP confined to 'Thai White' and 'Pink' guava died within nine and ten days, respectively. Logistic regression indicated that ACP longevity was longest on citrus and that longevity of ACP was longer on 'Pink' guava than on 'Thai White' guava (Table 7). These results are agreement with a report by Hall et al. (2008) showing that ACP did not survive for very long when confined to five different guava cultivars in a no-choice situation, with 95–100% mortality occurring within 6–9 days. These results indicated that, while guava may have traits that are repellent to ACP and although ACP may be able to sustain itself on guava for at least several days, guava did not appear to be acutely toxic to ACP. No oviposition occurred on guava during the test although young flush leaves were present.

Reports in the literature indicated intercropping guava and citrus can reduce the incidence of HLB in citrus by suppressing infestations of ACP, and a number of research efforts have been made to document this effect by guava on ACP and to discover reasons responsible (Hall et al., 2008; Rouseff et al., 2008; Gottwald et al., 2010; Zaka et al., 2010; Onagbola et al., 2011). The research presented here confirms that guava leaves have repellency to ACP and is the first study to show that extracts of oil from the leaves of five different guava cultivars have repellent activity. One of the cultivars, 'J3', is known to have repellency to *T. limbata*, a psyllid that

causes severe damage to some guava cultivars in Central and South America (Sá and Fernandes, 2015).

Reasons why guava exhibits repellency to ACP remain unclear. Rouseff et al. (2008) and Onagbola et al. (2011) hypothesized that sulfur compounds associated with guava, particularly dimethyl disulfide, were at least partially responsible. This compound is an insect toxic, defensive volatile produced only by guava leaves with injuries (crumpled or torn). For our studies on guava oil, we chopped leaves and then performed the *extractions*. We did not detect any sulfur compounds in the oil extracts (hydro-distillation extractions may miss some compounds such as sulfur) thus their repellency to ACP was the result of some other volatile (s). We found that both young and mature guava leaves repelled ACP, with mature leaves generally more repellent than young leaves.

Overall based on the results of this project, we conclude that guava does contain traits repellent to ACP, that leaf age plays some role in this repellency, and that there can be at least moderate differences in repellency among different guava cultivars.

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