Responses of the Asian Citrus Psyllid to Volatiles Emitted by the Flushing Shoots of Its Rutaceous Host Plants

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ABSTRACT *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) carries *Candidatus liberibacter* spp., the putative causal agents of Huanglongbing. *D. citri* reproduces and develops only on the flushing shoots of its rutaceous host plants. Here we examined whether *D. citri* is attracted to host plant odors and a mixture of synthetic terpenes. Tests conducted in a vertically oriented Y-tube olfactometer showed that both males and females preferentially entered the Y-tube arm containing the odor from the young shoots of *Murraya paniculata* (L.) Jack and *Citrus limon* L. Burm. f. cultivar Eureka. Only males exhibited a preference for the odor of *C. sinensis* L., whereas the odor of *C. × paradisi* MacFadyen cultivar Rio Red was not attractive to both sexes. The volatiles emitted by young shoots of grapefruit cultivar Rio Red, Meyer lemon (*Citrus × limon* L. Burm.f.), and *M. paniculata* were analyzed by gas chromatograph–mass spectrometry. The samples were comprised of monoterpenes, monoterpene esters, and sesquiterpenes. The number of compounds present varied from 2 to 17, whereas the total amount of sample collected over 6 h ranged from 5.6 to 119.8 ng. The quantitatively dominant constituents were (E)-β-ocimene, linalool, linalyl acetate, and β-caryophyllene. The attractiveness of a mixture of synthetic terpenes, modeled on the volatiles collected from *M. paniculata*, was evaluated in screened cages in a no-choice test. At three observation intervals, significantly more individuals were trapped on white targets scented with the mixture than on unscented targets. These results indicate the feasibility of developing *D. citri* attractants patterned on actual host plant volatiles.

KEY WORDS Hemiptera, kairomone, leaf volatiles, Huanglongbing, citrus

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is a phloem-feeding insect that is oligophagous on rutaceous host plants. Asian citrus psyllid is the primary vector in citrus of the bacteria *Candidatus liberibacter asiaticus* and *Candidatus liberibacter americanus* (Halbert and Manjunath 2004, Hung et al. 2004). These bacteria are assumed to be the causal agents of Huanglongbing or citrus greening disease, one of the most serious threats to citriculture worldwide (Bové 2006). Because Asian citrus psyllid is oligophagous on a variety of rutaceous host plants, such as the widely planted ornamental *Murraya paniculata* (L.) Jack, the range of Asian citrus psyllid has rapidly expanded into citrus production areas around the world (Halbert and Manjunath 2004, Bové 2006). Because of its occurrence in a variety of areas outside of orchards, such as residential areas (so-called dooryards), parks, schools, and commercial areas landscaped with *Murraya* and *Citrus,* as well as abandoned orchards, new tools are needed to improve its detection and monitoring.

Asian citrus psyllid mates, oviposits, and develops exclusively on new flush shoots; thus, its life cycle is closely tied to the growing pattern of its host plants (Moran and Buchan 1975, Hall and Albrigo 2007, Wenninger and Hall 2007, Sétamou et al. 2008). Recent studies have shown that stimuli emitted by flushing shoots may play an important role in the detection, location, and evaluation of potential host plants by Asian citrus psyllid. In terms of visual cues, Asian citrus psyllid is innately attracted to bright yellow and green (Sanchez 2008, Wenninger et al. 2009), colors that may be indicative of flushing shoots. Asian citrus psyllid is also stimulated by olfactory cues emitted by its host plants. Wenninger et al. (2009) recently showed Asian citrus psyllid responded to the odor of several host plants in a Y-tube olfactometer and that its antennae detected foliar volatiles. They also showed that the presence of olfactory cues enhanced the psyllids' response to visual cues in the Y-tube, indicating that Asian citrus psyllid can integrate information from at least two different sensory modalities during host plant search. The ability to correlate odor with color cues may facilitate a phytophagous insect's ability to recognize the overall stimuli signature from its host plant and differentiate it from surrounding vegetation (Patt & Sétamou 2007). Olfactory cues have been

shown to be important in host plant location in other psyllid species (Moran and Brown 1973, Lapis and Borden 1993, Soroker et al. 2004, Horton and Landolt 2007).

In this study, we conducted a preliminary investigation of the volatile compounds emitted by the young shoots of representative Asian citrus psyllid host plants. Analyses were performed on the odors collected from grapefruit (*Citrus × paradisi* Macfadyen) cultivar Rio Red, Meyer lemon (*Citrus × limon* L. Burm.f.), and orange jasmine. Asian citrus psyllid commonly occurs on all three plants in the lower Rio Grande Valley (M.S., unpublished data). We also evaluated the response of male and female Asian citrus psyllids to the odor of flushing host plants in a Y-tube olfactometer. Last, we conducted a preliminary experiment to evaluate the response of Asian citrus psyllid in screened cages to a mixture of synthetic terpenes that was representative of the volatiles collected from orange jasmine. The overall aim of this study was to identify potential volatile compounds that could be used to develop olfactory lures for enhancing Asian citrus psyllid detection and monitoring.

Materials and Methods

Y-Tube Olfactometer. Olfactometry tests were performed in a glass Y-tube olfactometer (model OLFM-ADS-4AFN2C; ARS, Gainesville, FL) with Teflon tubing and connections. The Y-tube was oriented vertically with arms situated 30 cm below fluorescent lights. An air pump (KNF Laboratories, Trenton, NJ) was used to provide airflow, and a 1,000-ml Erlenmeyer flask with 500 ml of distilled water was used to provide humidity to the system. Test aromas originated from single sprigs of young shoots (Hall and Albrigo 2007) (≈10–12 cm long) placed within a glass flask (ARS) connected to the airline system. To maintain their turgor, the cut ends of the sprigs were placed in capped florist tubes (Floral Suppliers Syndicate, Camarillo, CA) filled with 20 ml of 10–5-14 (N-P-K) hydroponic solution (MaxiGro; General Hydroponics, Sebestopol, CA). Aroma from the sprigs passed through one Y-tube arm, whereas purified, blank air passed through the other arm. Aromas from Rio Red grapefruit, sweet orange (*C. sinensis* L.), lemon (*Citrus limon* L. Burm. f.) cultivar Eureka, and orange jasmine were tested. Asian citrus psyllids were obtained from a laboratory colony maintained on potted orange jasmine, Meyer lemon, and sweet orange. Asian citrus psyllids were sexed before the experiment and tested individually. After its introduction into the olfactometer base, each Asian citrus psyllid was given 300 s to crawl into one of the Y-tube arms and was scored responsive if it entered either arm. Psyllids had to remain in a particular arm for ≥60 s to be scored as having made a selection. Individuals that did not enter at least one of the arms were scored as unresponsive. Asian citrus psyllid were tested until at least 30 responded per host plant. Tests were conducted between 1300 and 1600 hours, with illumination provided by overhead fluorescent lights at 900 lux,

ambient temperature of 24–27°C, and 50–65% RH. The arms of the Y-tube were reversed after five psyllids were tested to prevent directional bias. After testing, the entire Y-tube and its components were washed with hot water and detergent, rinsed with 95% ethanol, and air dried. *G*-tests with Yates' correction were performed to compare the numbers of Asian citrus psyllids selecting the different arms of the Y-tube olfactometer (Zar 1999).

Before the tests with host plant odors, preliminary tests were conducted to determine whether the two arms of the Y-tube olfactometer were balanced. Adult females were starved for 16–20 h by keeping them in tubes maintained at 26°C in an incubator. During the test, both arms of the olfactometer were kept blank. Choice tests were conducted with starved females in olfactometer until a total number of 10 female Asian citrus psyllids selected either arm of the olfactometer. The insects were given 10 min to make a choice. Only insects entering either arm of the olfactometer were considered responsive and included in the analysis. After testing five insects, the arms of the olfactomer were rotated until the required number of 10 responding individuals was obtained. A χ^2 test was used to compare the number of adults responding to either arm of the olfactometer.

Flush Odor Collection and Analysis. Volatiles from the young shoots of orange jasmine, Rio Red grapefruit, and Meyer lemon were collected with the headspace-purge and trap method. The plants used for the collection were grown in 3-gal pots in standard potting mix and were 3–4 yr of age. All plants used in the collections originated from the Texas A & M University Citrus Center in Weslaco, TX. Collections were made in the USDA–ARS–KSARC greenhouse at 27 ± 2°C. At the start of each collection, the terminal portion of the shoot was gently inserted into the center neck of a 100-ml three-necked glass flask. The shoots were between 0.5 and 2.5 cm in length. Samples were collected for 6 h (0800–1400 hours) at a flow rate of 100 ml/min with sorbent tubes (425 mg Carbotrap; Supelco, Bellefonte, PA). Cotton wadding was used to seal the middle neck of the flask. Make-up air for the sample was introduced at 100 ml/min from a pressurized cylinder of purified air (Zero Grade Air; Acetylene-Oxygen, Harlingen, TX) connected to a flow regulator with charcoal filters (model VCS-ADS-4AFM4C; ARC). Control collections taken from empty glass flasks showed no background air contaminants.

Immediately after collection, the trap tubes were eluted with 3 ml of diethyl ether. The diethyl ether contained toluene as an internal standard (2.5 μl of toluene in 100 ml diethyl ether). After elution, the sample was further condensed to 100 μl under a stream of nitrogen. One microliter of condensate was injected into an Agilent 6890 Series gas chromatograph equipped with a mass selection detector under the following conditions: injector: 250°, splitless mode; column: 60 m by 0.25 mm DB-1 methyl silicon (J & W Scientific, Santa Clara, CA), film thickness 0.25 μm; temperature program: 50°C (2 min) and then 5°C/min to 220°C (10 min). The mass spectra detector param-

eters were as follows: transfer line temperature 280°, mass range scanned 20–400 mass units, source temperature 280°, MS quad temperature 280°, and EM volts 2,188.

Spectral matching was accomplished with ChemStation software (Agilent, Santa Clara, CA) and the NIST 98 Mass Spectral Library (v 1.7) and Essential Oil Components Library (Adams 2007). A positive matched required a spectral fit of ≥90. Verification of compound identification was obtained by comparison of their spectra to the retention times and mass spectra of standard compounds (commercial sources of standard compounds are listed in Tables 2–4). In cases where commercially available standard compounds were not readily available, natural product essential oils known to contain the compounds in question (Dugo et al. 2002) were used to verify the retention times and mass spectra of sample components. Essential oils (Ananda, Boulder, CO) were used to verify the following compounds: petigrain oil, β-elemene; sweet orange peel oil, neral and δ-cadinene; grapefruit peel oil, germacrene D and copaene; lemon peel oil, bergamotene; ginger oil, α-zingiberene. Essential oil samples were made by mixing 1 μl of essential oil in 100 ml diethyl ether followed by evaporation under nitrogen to 100 μl. An estimate of the concentration of individual components was determined by comparison of their peak area with that of the internal standard (toluene) added to the elutant.

Test of Synthetic Terpene Mixture. A proprietary mixture of synthetic terpenes was formulated based on the odor emitted by the young shoots of orange jasmine, which was chosen as a model because of the relative simplicity of its volatile composition (see Results) and its importance as an Asian citrus psyllid host plant (Halberth and Munjunath 2004). The mixture contained six synthetic mono- and sesquiterpenes in proportions that approximated their proportions in the samples (a 5:3:1:1:1:1 mixture of β-ocimene, β-caryophyllene, linalool, α-cubebene, D-limonene, and myrcene; sources as listed in Tables 2–4).

The attractiveness of the mixture to Asian citrus psyllid was tested with no-choice tests with either scented or unscented visual targets. The tests were conducted in screened cages between 1230 and 1630 hours in the USDA–ARS–KSARC greenhouse at a temperature of 29 ± 1°C and a relative humidity of 60%. The cages measured 45 cm one each side and were covered with fine biege-colored nylon screen (BioQuip, Rancho Dominguez, CA). Vertically oriented white drinking straws were used as visual targets. White was selected as the target color to minimize the effect of color attraction on Asian citrus psyllid (Sanchez 2008). A pair of straws, which were either scented or unscented, was placed in each cage. The straws were supported by a plastic rack and were positioned 15 cm apart from each other, 8 cm from the cage back and ceiling, and 12 cm from the nearest cage side. The straws were covered with double-sided cellophane tape to trap any Asian citrus psyllids that alighted on the visual targets. Odor dispersal was accomplished by adding 5 μl of the flush volatile mixture

Table 1. Results of a Y-tube olfactometer test showing percentage of mean no. of responsive male and female Asian citrus psyllid and the percentage of each sex choosing the Y-tube arm with the host plant odor

Host plant	Sex (no. tested)	Percent responding	% respondents choosing scented arm
Rio red grapefruit	Male (30)	100	60[NS]
	Female (42)	71	46[NS]
Sweet orange	Male (37)	81	70[a]
	Female (34)	88	57[a]
Eureka lemon	Male (39)	77	87[a]
	Female (35)	73	73[a]
Orange jasmine	Male (35)	77	77[a]
	Female (43)	90	60[a]

[a] Significantly different at $P \leq 0.01$ (G-test).
NS, not significant.

to a cotton wick positioned at the top of each straw. The mixture was not added to the cotton wick in the unscented straws. The mixture was applied to the cotton wicks 15 min before the start of each test to permit diffusion of the volatiles within the cage. At the start of the test, 35 Asian citrus psyllid of mixed sex and age were released within each cage. The Asian citrus psyllid used in the tests were obtained 3–4 h previously from the Texas A&M University Citrus Center research orchard. The numbers of Asian citrus psyllids trapped on each straw were counted at 30-min intervals for a total of 90 min. A total of 14 replicates of each treatment were performed between 24 Oct. and 7 Nov. 2008. G-tests with Yates' correction were performed to compare the numbers of Asian citrus psyllids trapped by the scented and nonscented targets in the flush volatile mixture tests (Zar 1999).

Results

In the preliminary test with blank air, no significant differences were obtained between both arms of the olfactometer with 60 and 40% of insects entering arm 1 and arm 2, respectively (corrected χ^2 test = 0.19, df = 1; $P = 0.65$). This result showed that both arms of the olfactometer were equivalent and did not introduced any bias during choice tests with host plant flush shoots.

More than 70% of Asian citrus psyllids entered either arm of the olfactometer, indicating that most individuals tested were in a responsive condition (Table 1). Both males and females preferentially entered and remained in the arm containing the sprig odor from orange jasmine and Eureka lemon shoots over the unscented control. For sweet orange, only males entered the arm with sprig odor in significantly greater numbers than the control. In tests with Rio Red grapefruit, neither males nor females entered the sprig odor arm preferentially over the unscented arm. For all host plants tested, males showed a higher level of entering the arm with host plant odor than did females.

The odor samples were composed primarily of monoterpenes, monoterpene esters, and sesquiterpenes (Tables 2–4). Twelve volatile compounds

Table 2. Volatile compounds emitted by flushing shoots of orange jasmine (*M. paniculata*)

Compound	RT	Collection date (%)			
		Aug. 6	Aug. 18	Aug. 22	Aug. 29
(Z)-3-hexen-1-ol acetate[a]	18.60		1.6		
(Z)-β-ocimene[b]	19.92		1.6		
(E)-β-ocimene[b]	20.26	68.2	35.5	66.7	4.7
Linalool[b]	21.54	4.5	4.8		
δ-elemene[c]	28.36				7.5
Copaene[c]	29.40		1.6		3.3
α-cubebene[d]	29.62	4.5	4.8		10.8
β-caryophyllene[b]	30.32	18.2	35.5	33.3	53.1
α-caryophyllene[b]	30.93		3.2		6.1
Germacrene D[c]	31.37	4.5	6.5		10.8
α-zingiberene[c]	31.43		1.6		3.8
δ-cadinene[c]	31.93		3.2		
Total amount collected (ng)		11.8	15.6	1.7	16.1

The collection date, percent composition of volatiles, and total amount of volatiles collected are given for each sample.
[a] The commercial source is SAFC.
[b] The commercial source is Fluka.
[c] The commercial source is essential oil (Ananda).
[d] The commercial source is GFS Chemical.

were identified in samples collected from orange jasmine (Table 2). The primary constituents were the monoterpene (*E*)-β-ocimene and the sesquiterpene β-caryophyllene. The oxygenated monoterpene linalool and the sesquiterpenes germacrene D and β-cubebene were present in lower amounts. Minor constituents included the green leaf volatile (*Z*)-3-hexen-1-ol acetate and various sesquiterpenes. Total amounts of volatiles collected from orange jasmine during the 6-h sampling period ranged

Table 3. Volatile compounds emitted by flushing shoots of Meyer lemon

Compound	RT	Collection date (%)				
		Jul 16	Aug 14	Sep 10	Sep 22	Sep 23
β-pinene[a]	18.11	0.2	0.6			
β-myrcene[a]	18.47	1.7	3.5	5.9	2.8	3.3
3-carene[b]	19.25	0.2	1.2			0.3
D-limonene[b]	19.76	0.5	2.9	4.8	5.2	1.3
(Z)-β-ocimene[a]	19.87	0.5	1.2	2.9		1.2
(E)-β-ocimene[a]	20.20	2.9	4.7	10.3	1.7	7.0
Terpinolene[b]	21.39	0.2	0.6	1.3		0.3
Linalool[a]	21.48	27.3	40.1	44.4	41.2	33.4
Allo-ocimene[a]	22.33	0.2	0.6			0.4
α-terpineol[a]	23.78					0.1
Neral[c]	24.81					0.1
Linalyl acetate[b]	25.46	59.5	43.6	28.4	49.1	43.7
Gernial[b]	25.57	0.7		0.4		1.0
Neryl acetate[a]	28.35			0.8		1.2
Geranyl acetate[d]	28.78	2.0				4.0
β-elemene[c]	29.58					
β-caryophyllene[a]	30.26	2.9	1.2	1.0		2.5
α-caryophyllene[a]	30.86	0.5				
Total amount (ng)		159.2	52.3	39.1	22.2	51.4

The collection date, percent composition of volatiles, and total amount of volatiles collected are given for each sample.
[a] The commercial source is Fluka.
[b] The commercial source is SAFC.
[c] The commercial source is essential oil (Ananda).
[d] The commercial source is Sigma- Aldrich.

Table 4. Volatile compounds emitted by flushing shoots of Rio red grapefruit

Compound	RT	Collection date (%)			
		Aug. 12	Aug. 26	Sept. 12	Oct. 15
α-pinene[a]	16.56	0.6			
Sabinene[b]	17.92	11.9	15.2		0.4
β-pinene[a]	18.11				1.1
β-myrcene[a]	18.48	20.2			
(Z)-3-hexen-1-ol acetate[c]	18.59	0.8			
D-limonene[c]	19.80	15.9	9.1	3.9	0.3
(Z)-β-ocimene[a]	19.92	0.8			2.3
(E)-β-ocimene[a]	20.26	35.5	75.8	96.1	89.7
Linalool[a]	21.54	2.8			0.3
Allo-ocimene[a]	22.39				1.1
Neo-allo-ocimene[a]	22.70				0.3
α-terpineol[c]	23.78	0.8			
Neral[d]	24.81	1.1			
Geranial[c]	25.54	1.9			
α-copaene[d]	29.39				0.1
β-elemene[d]	29.62	3.0			
α-bergamotene[d]	30.07				1.8
β-caryophyllene[a]	30.32	1.7			1.8
β-farnesene[e]	30.69	0.8			
Unidentified	30.78				0.7
α-caryophyllene[a]	30.93	0.4			
δ-cadinene[d]	31.93	0.8			
Total amount (ng)		119.8	8.5	5.6	71.3

The collection date, percent composition of volatiles, and total amount of volatiles collected are given for each sample.
[a] The commercial source is Fluka.
[b] The commercial source is Berge.
[c] The commercial source is SAFC.
[d] The commercial source is essential oil (Ananda).
[e] The commercial source is IFF.

from 1.7 to 15.6 ng. Eighteen volatile compounds were identified in the samples collected from Meyer lemon (Table 3). All of the samples contained large proportions of linalool and linalyl acetate. Minor constituents included β-caryophyllene, (*E*)-β-ocimene, and β-myrcene. Total amounts of volatiles collected from Meyer lemon during the 6-h long sampling period ranged from 22.2 to 149.2 ng. Twenty-two volatiles were identified in samples collected from Rio Red grapefruit (Table 4). In all samples, (*E*)-β-ocimene was the dominant compound. The more abundant secondary compounds included D-limonene, β-myrcene, and sabinene. Total amounts of volatiles collected from Rio Red grapefruit during the 6-h sampling period ranged from 5.6 to 119.8 ng.

In the screen cage assay, adult Asian citrus psyllids were responsive to a mixture of six synthetic terpenes formulated in proportions that approximated their abundance in the odor samples collected from orange jasmine. At each 30-min exposure interval, significantly more Asian citrus psyllids were trapped on the scented targets than on the unscented targets (Table 5). A maximum of 21 and 14% of the total psyllids were trapped, respectively, in the scented and unscented treatments.

Discussion

Our results show that Asian citrus psyllid is attracted by volatiles emitted by the growing shoots of various

Table 5. Mean ± SEM no. Asian citrus psyllids captured on scented and unscented white targets at 30-min intervals in a screened cage

Trap treatment	Exposure time (min)		
	30	60	90
Unscented	1.3 ± 0.3a	2.6 ± 0.5a	4.6 ± 1.7a
Scented	2.6 ± 0.7b	4.4 ± 0.9b	7.6 ± 1.4b

Thirty-five Asian citrus psyllids were released per cage per test with a total of 14 replicates made for each treatment. Numbers within the same column with different letters are significantly different at $P < 0.05$ (*G*-test).

citrus species and orange jasmine. Furthermore, the results indicate that it may be possible to formulate scent attractants for Asian citrus psyllid based on the volatile profiles of the flushing stages of its host plants. The samples collected from the flushing shoots were mixtures of primarily monoterpenes, monoterpene esters, and sesquiterpenes, all of which commonly occur in the foliage of *Citrus* species (Dugo et al. 2002, Lota et al. 2002, Gancel et al. 2003). Each species had a general odor profile in which one or two volatile constituents were quantitative codominants with a few compounds present in lesser amounts. Analyses of essential oils derived from the leaves of various *Citrus* species and their hybrids have shown the presence of distinct chemotypes, in which one to several compounds are found to be qualitatively dominant (Lota et al. 2002, Gancel et al. 2003). It may be possible that such chemotypes are also relevant to the profiles of volatiles emitted by the foliage.

There was an unexpectedly high degree of variation in the number and quantity of volatiles emitted by the shoots used in the study. This high level of variation is difficult to understand, because all of the plants were grown together, watered and fertilized on the same schedule, and sampled in the greenhouse. One possible explanation for the observed variation may have been because of the age of the shoots. That is, even though the shoots had similar lengths, some were older than others because shoots on different plants did not grow at the same rate.

Host finding behavior in psyllids is complex and involves different sensory modalities and behavioral and physiological states. During host plant search, psyllids may use volatiles cues from both host plants and conspecifics (Moran and Brown 1973, Lapis and Borden 1993, Scutareanu et al. 2003, Soroker et al. 2004, Horton and Landolt 2007, Wenninger et al. 2008). Asian citrus psyllid responsiveness to the odor emitted by flushing shoots may depend on a number of different odor characteristics, such as volatile composition, proportionality or ratio, and concentration (Bergström et al. 1995). For example, it is not clear why Asian citrus psyllids were not more attracted to Rio Red sprigs in the Y-tube tests because they can be abundant in Rio Red orchards. Perhaps contextual cues are more important in host plant location for some Asian citrus psyllid host plants than in others.

Based on the observation that the flush odors are comprised of codominating volatiles constituents, we speculate that mixtures of two or more flush volatiles may act synergistically and be more stimulatory to Asian citrus psyllid than individual compounds. When mixed in particular proportions, certain volatile mixtures have been shown to be highly synergistic with respect to attracting phytophagous insects (Natale et al. 2003, Ruther and Mayer 2005, Bengtsson et al. 2006, Pinero and Dorn 2007). Wenninger et al. (2009) found the antennal response of Asian citrus psyllid to synthetic limonene was somewhat lower than to the entire suite of volatiles emitted by grapefruit foliage, indicating that Asian citrus psyllids may be more responsive to blends than to individual compounds. At this early point in the study, of Asian citrus psyllid response to host plant volatiles, we cannot yet explain the nature of Asian citrus psyllid's response to the synthetic terpene mixture. The greater number of psyllids trapped on the scented targets could be caused by several types of responses, such as an increase in general stimulation, positive chemotaxis, or an increased attraction to visual stimuli.

It is also possible that some minimum number of compounds is needed to attract Asian citrus psyllids. A synergism between ubiquitous foliar volatiles and more specialized foliar volatiles is thought to give rise to the attractiveness of particular plant volatile mixtures to herbivores (Visser 1986, Harrewijn et al. 1995). A combination of at least five compounds was needed to elicit an orientation response in female oriental fruit moth, *Cydia molesta* Busck, which oviposits on the flushing shoots of peach (Pinero and Dorn 2007). In this case, a combination of three particular green leaf volatiles and two aromatic compounds emitted by peach flush was needed for the mixture to be attractive to the moth. Pinero and Dorn (2007) hypothesized that attraction of the synthetic mixture was caused by a combination of ubiquitous foliar volatiles (i.e., green leaf volatiles) and specific foliar volatiles (i.e., a nitrogen-containing aromatic compound).

Citrus foliar volatiles are comprised primarily of common monoterpenes, sesquiterpenes, and monoterpene esters. Because Asian citrus psyllids can occur on a wide variety of chemically diverse species, its searching behavior may be influenced by the aggregate presence of volatiles from two or three of the chemical classes of citrus volatiles as a whole. Thus, the initial synthetic mixture tested here contained components from two of the three chemical groups: monoterpenes and sesquiterpenes. The apparent prevalence of *Citrus* chemotypes, combined with the concept of odor attractiveness attributed to the presence of common and specific volatiles, leads us to speculate that Asian citrus psyllid attractants will need to contain general foliar monoterpenes, such as linalool or limonene, and foliar volatiles that are prevalent in the Rutaceae, such as sesquiterpenes or monoterpene esters. The codominant compounds found in our samples, such as (*E*)-β-ocimene, linalool, linalyl acetate, and β-caryophyllene, may be especially important with respect to signifying the presence of flush to Asian citrus psyllids, but more extensive sampling is

needed to ascertain this idea. The presence of volatiles that also signify the presence of young shoot growth, such as CO_2, ethylene, and cuticle precursors, may also synergize the response of Asian citrus psyllid to the terpenes emitted by flush (Harrewijn et al. 1994, Goyret et al. 2008).

Volatile concentration may also be an important factor in determining whether particular compounds or mixtures are stimulatory. Electroantennogram (EAG) and morphological studies suggest that psyllids can accommodate high concentrations of volatiles when their host plants have glandular foliage (Kristoffersen et al. 2006, Onagbola et al. 2008, Wenninger et al. 2009). In our tests, Asian citrus psyllids responded to a mixture of undiluted standard compounds within the confines of a screened cage. However, Lapis and Borden (1993) found that *Heteropsylla cubana* Crawford did not orient to highly concentrated extracts of host plant leaves. Further studies with graduated concentrations of individual compounds and mixtures are needed to determine the relationship between volatile concentration and Asian citrus psyllid response levels.

The findings of Yasuda et al. (2005) and Wenninger et al. (2009), along with the results presented here, indicate that male and female Asian citrus psyllid respond differentially to host plant odor. Sex-based responses to host plant odor have been observed in other psyllids (Lapis and Borden 1993, Soroker et al. 2004, Horton and Landolt 2007), and Wenninger et al. (2009) also showed that host plant selection by Asian citrus psyllids is influenced by mating status. Interestingly, in our study, Asian citrus psyllids displayed attraction to three of four host flush odors in the absence of visual cues within the Y-tube olfactometer. This is contrary to the results obtained by Wenninger et al. (2009), who observed very minimal attraction to host plant odors in a Y-tube in the absence of a strong visual cue. The discrepancy in the results of the two studies may be a consequence of the orientation of the Y-tubes. The Y-tube in Wenninger et al. was oriented horizontally, whereas the Y-tube used in this study was oriented vertically. In a vertically oriented Y-tube, the psyllids' phototaxic response may have been influenced by the presence of olfactory cues. Similarly, within the confines of the Y-tube, psyllids may have been more inclined to respond to olfactory cues (in the absence of visual cues) while they were engaged in phototaxis. Evidence for the interaction between phototaxis and olfaction was seen by M.S. (unpublished data), who observed only a limited response by Asian citrus psyllids to host plant odors in a horizontally oriented Y-tube and found that Asian citrus psyllid does not respond significantly to host plant odors in Y-tubes in the absence of light. Strong positive phototaxis may also partially explain why relatively few Asian citrus psyllids were trapped in the synthetic scent tests because the tops of the targets were situated 8 cm below the cage ceiling and approaching the targets required the psyllids to fly downwards. Yasuda et al. (2005) observed Asian citrus psyllid behavior on inverted *Murraya exotica* plants and noted that males

appeared to orient to the tips of flushing shoots primarily by positive phototaxis. In other pysllid–host plant systems, younger foliage infested with pysllids releases volatiles that are different from older infested foliage (Scutareanu et al. 1997). Combined, these findings indicate that odor-mediated phototaxis may help Asian citrus psyllids to locate new flushing shoots located within the same tree.

In addition to above-mentioned factors, life history, seasonal plant phenology, and infection of host plants by pathogens may also influence the Asian citrus psyllid response to host volatiles. During the dispersal phase of their life cycles, certain psyllids may become highly attracted to single compounds, as in the case of *Cacopsylla picta* Foerster and β-caryophyllene (Mayer et al. 2008a, 2008b). Interestingly, in this case, a phytoplasma vectored by *C. picta* induced the release of the β-caryophyllene from infected host plants. Some aphids also respond differentially to the odors emitted by host plants infected with pathogens that they vector (Eigenbrode et al. 2002, Ngumbi et al. 2007). It is possible that Asian citrus psyllid responds differentially to the odor emitted by its host plants when they are infected with Huanglongbing.

Acknowledgments

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