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VOLATILE PROFILES OF YOUNG LEAVES OF RUTACEAE SPP. VARYING IN SUSCEPTIBILITY TO THE ASIAN CITRUS PSYLLID (HEMIPTERA: PSYLLIDAE)

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The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) was first reported in Florida by Halbert (1998). Halbert (2005) also reported the discovery in Florida of the devastating citrus disease, citrus greening, whose pathogen (*Candidatus* Liberibacter asiaticus) it vectors (Gottwald et al. 2007). An understanding of the role that plant volatiles play in host plant finding by *D. citri* would be useful in formulating insect management strategies as well as aiding in the production of resistant or tolerant cultivars through both traditional breeding and genetic engineering. Using a Y-tube olfactometer, recent work (Patt & Sétamou 2010) demonstrated the response of *D. citri* to shoots of various citrus species as well as synthetic volatiles identified by gas chromatography-mass spectrometry (GC-MS) from those species. Wenninger et al. (2009) indicated that *D. citri* is responsive to host plant volatiles and that the response varies by mating status, plant species and the presence or absence of visual cues. Using both behavioral and electroantennographic bioassays, Soroker at al. (2004) demonstrated that adult female pear psyllids, *Cacopsylla bidens* (Šulc), respond to odors of their pear host. Early work by Moran & Brown (1973) on *Trioza erytreae* (Del Guercio), an African citrus-feeding psyllid, illustrated that antennal chemoreception of volatile plant secondary compounds was important but that antennal tip gustation might also be involved.

Nymphs of *D. citri* require the newly expanding terminal leaves (flush) of citrus and related species in order to complete their development (Shavankar et al. 2000; Michaud 2004). The study presented here examined host plant volatiles from uninfested flush and young leaves of citrus genotypes that were reported to vary in susceptibility as measured by colonization by *D. citri* in the field (Westbrook et al. 2011). Five species of Rutaceae were selected for plant volatile analysis from a list of genotypes assayed for colonization by Westbrook et al. (2011). In that study, 87 genotypes were examined in the field for presence of eggs, nymphs and/or adults. We collected and analyzed volatiles from 3 genotypes on which adults were abundant, including *Bergera koenigii* L. (curry leaf tree), *Murraya paniculata* (L.) Jack (orange jasmine) and *Citrus macrophylla* Wester. Volatiles were also analyzed from *C. jambhiri* Lush. ('Rough' lemon), a genotype on which the *D. citri* population was nearly midway between the highest and lowest recorded, and from *Poncirus trifoliata* L. (trifoliate orange), whose population of adults was near the lowest recorded in the study. *C. sinensis* (L.) Osbeck 'Valencia' was also included because of its widespread commercial use and susceptibility to *D. citri*.

Plant volatiles were collected by placing freshly excised 15 g bouquets consisting of shoots of terminal leaves and flush of each genotype (≤ 7 d old) into separate 50 ml beakers containing distilled water. Bouquets from *B. koenigii* were collected from field-grown plants whereas bouquets from the other 5 genotypes were collected from potted greenhouse-grown plants maintained at 22.2 to 26.7 °C under natural light conditions. The beaker was then placed into a closed ca. 1-liter glass container. Charcoal-filtered humidified air was pushed through the container at a rate of 500 ml/min for 24 h. Plant volatiles were trapped in glass tubes packed with Porapak Q (#226-115, SKC Inc., Eighty Four, PA). Volatiles were eluted from the tubes with 2 ml methylene chloride. The solvent was evaporated under a gentle stream of N_2 to a volume of 0.5 ml. From this, 1 µl was used for GC-MS analysis.

The eluates were analyzed by GC-MS using a ThermoElectron Trace GC coupled to a DSQII quadrupole MS, all controlled by Xcalibur software. The GC-MS system was equipped with a PTV injector port run in the PTV mode and a 30m × 25mm (ID) × .25µm film DB5MS (Agilent, Santa Clara, CA) fused silica open tubular column, using helium (99.999% pure) for the carrier gas. Compounds were identified by using both the Retention Index (RI) of the eluted peaks and the background-subtracted mass spectrum averaged across the total ion chromatogram peak or across a characteristic extracted ion current peak when co-elution of multiple compounds was detected. The RI database was generated at USHRL using the same column and GC-MS system by injecting synthetic standards or volatile collections and calculating the RI based on the retention times of a mixture of alkanes. Mass spectra were compared to those recorded in the NIST Library (2005). For identification purposes, RIs had to match within

TABLE 1. PERCENTAGES OF COMPOUNDS FOUND IN HEADSPACE VOLATILES COLLECTED FROM UNINFESTED FLUSH AND YOUNG LEAVES OF VARIOUS RUTACEAE.

B. koenigii	%
β–phellandrene	48.3
β–caryophyllene	27.2
α-pinene	6.0
α-humulene	3.9
α-phellandrene	2.9
(*E*)- β-ocimene	2.4
(*E*)-α-bergamotene	1.5

C. sinensis 'Valencia'	%
sabinene	46.3
(*E*)- β-ocimene	14.0
Δ^3-carene	6.9
limonene	5.0
linalool	3.8
$C_{10}H_{16}$	2.6
β-pinene	2.2
α-pinene	1.7
β-caryophyllene	1.4
β-cubebene	1.2

C. jambhiri	%
limonene	55.4
sabinene	13.0
(*E*)- β-ocimene	10.1
β-caryophyllene	8.5
(*E*)-α-bergamotene	2.3
1,8-cineole	2.0
β-myrcene	1.6

M. paniculata	%
germacrene-D	11.5
pentocosanal	9.2
β-caryophyllene	7.8
β-cubebene	7.4
α-zingiberene	4.4
α-copaene	3.2
2-ethyl hexanol	2.6
2,2-dimethylindane	2.5
(*Z*)-3-hexenyl acetate	2.5
phytane	2.1
decanoic acid ester	1.9
UNKNOWN	1.5
α-humulene	1.4
(*E*)-2-hexenal	1.4
p-ethylacetophenone	1.3
δ-cadinene	1.3
α-cucurmene	1.3
N,N-dimethylstearylamine	1.2
UNKNOWN	1.2
UNKNOWN	1.2
UNKNOWN	1.1
nonanal	1.1
phenoxypropanol	1.1
nonadecane	1.0

C. macrophylla	%
limonene	60.7
p-cymene	5.5
geranial	3.2
neral	3.0
nerol	2.8
carvone and geraniol	2.2
UNKNOWN	2.2
(+)-(*Z*)-1,2-limonene epoxide	1.9
linalool	1.4
(+)-(*E*)-1,2-limonene epoxide	1.4
δ-elemene	1.4
$C_{10}H_{16}$	1.2
caryophyllene oxide	1.2

P. trifoliata	%
β-myrcene	7.7
1-octadecene	7.5
hexadecene	7.2
germacrene-D	6.8
(*E*)- β-ocimene	6.4
$C_{15}H_{22}O$	5.5
1-eicosene	3.0
decanal	2.9
2-ethyl hexanol	2.6
UNKNOWN	2.4
β-caryophyllene	2.2
UNKNOWN	2.0
decanoic acid ester	1.9
p-ethylacetophenone	1.9
indole	1.8
1-tetradecene	1.6
diacetone alcohol	1.6
2-methyl-6-methylene-1,7-octadiene-3-one	1.6
heptadecane	1.6
geranylacetone	1.6
hexadecane	1.5
UNKNOWN	1.4
nonanal	1.4
β-bisabolene	1.3
$C_{15}H_{26}O$	1.2
(*Z*)-3-hexenyl acetate	1.1
tetradecane	1.1
UNKNOWN	1.1
UNKNOWN	1.1
$C_{10}H_{12}O$	1.1
3,5-di-tert-butyl-4-hydroxybenzaldehyde	1.1
tridecane	1.0

5 RI units of the average database value and the reverse search of the sample spectrum had to achieve a match of > 500 of 1000.

Percentages of volatile compounds found in amounts ≥ 1% are reported for each of the 6 genotypes in Table 1. Limonene and (*E*)-β-ocimene were the only volatiles that occurred in the headspace over all 6 genotypes. Whereas limonene was present at high percentages in headspace collected from *C. jambhiri* and *C. macrophylla* collections (55.4% and 60.7%, respectively), it constituted 5.0% of volatiles from *C. sinensis* 'Valencia' and < 1% (not shown) from *B. koenigii*, *M. paniculata* and *P. trifoliata*. Similarly, (*E*)-βocimene was found at levels of 10.1% and 14.0% in *C. jambhiri* and *C. sinensis* 'Valencia' headspace, respectively, but only at 2.4% of volatiles collected from *B. koenigii*; < 1% of this compound was found in extracts of *C. macrophylla*, *M. paniculata* and *P. trifoliata* headspace.

The headspace collection from *B. koenigii*, a genotype among those with the highest counts of adults, nymphs and eggs in the Westbrook et al. (2011) study, contained 48.3% ß-phellandrene. Rajeswara Rao et al. (2011) reported titers of ß-phellandrene in *B. koenigii* that varied from 14.7 - 50.2% across 10 locations in India. Other studies analyzing volatiles from the same and closely related citrus genotypes reported results similar to those detailed here (Lota et al. 2002, Gancel et al. 2003, Patt & Sétamou 2010)

Enumeration of the volatiles in various rutaceous genotypes does not explain their role in *D. citri* attraction, repellency, settling or growth. It does, however, suggest a subset of likely candidates for evaluation in behavioral assays. These choices can be made on the basis of the high titers of compounds found in attractive genotypes such as the sabinene in *C. sinensis* 'Valencia' or the ß-phellandrene in *B. koenigii* or in commonly shared compounds such as the large percentages of limonene found in *C. jambhiri* and *C. macrophylla*. In genotypes such as *P. trifoliata*, it will be important to assess whether it is a lack of key attractive components that renders it unsuitable for *D. citri* or whether it is the presence of repellent or toxic compounds. Behavioral analysis of identified compounds may produce useful information for psyllid management and plant breeding purposes.

Summary

This note reports the proportions of various compounds found in the headspace volatiles among 6 species in the family Rutaceae differing in their susceptibility to colonization by the Asian citrus psyllid. Some compounds occurred exclusively while others were common to several species. Behavioral analysis of individual compounds as well as blends of compounds may produce useful information for psyllid management and plant breeding strategies.

References Cited

Gancel, A.-L., Ollitrault, P., Froelicher, Y., Tomi, F., Jaquemond, C., Luro, F., and Brillouet, J.-M. (2003). Leaf volatile compounds of seven citrus somatic tetraploid hybrids sharing willow leaf mandarin (*Citrus deliciosa* Ten.) as their common parent. J. Agric. Food Chem. 51: 6006-6013.

Gottwald, T. R., da Graça, J. V., and Bassanezi, R. B. 2007. Citrus huanglongbing: The pathogen and its impact. On line. Plant Health Progress doi: 10.1094/PHP-2007-0906-01-RV.

Halbert, S. E. 1998. Entomology section. Tri-ology 37: 6-7.

Halbert, S. E. 2005. The discovery of huanglongbing in Florida. Proc. of the Second International Citrus Canker and Huanglongbing Research Workshop. Florida Citrus Mutual, Orlando, FL, 7-11 Nov., Paper H-3. p. 50.

Lota, M.-L., de Rocca Serra, D., Tomi, F., Jaquemond, C., and Casanova, J. (2002). Volatile components of peel and leaf oils of lemon and lime species. J. Agric. Food Chem. 50: 796-805.

Michaud, J. P. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. Biol. Cont. 29: 260-269.

Moran, C. V., and Brown, R. P. 1973. The antennae, host plant chemoreception. and probing activity of the citrus psylla, *Trioza erytreae* (Del Guerico) (Homoptera: Psyllidae). J. Entomol. Soc. S. Afr. 36: 191-202.

NIST/NIH/EPA. Mass Spectral Library, Standard Reference Database 1, NIST 05. Standard Reference Data Program, National Institute of Standards and Technology, Gaithersburg, MD, USA, 2005.

Patt, J. M., and Sétamou, M. 2010. Responses of the Asian citrus psyllid to volatiles emitted by the flushing shoots of its rutaceous host plants. Environ. Entomol. 39: 618-624.

Rajeswara Rao, B. R., Rajput, D. K., and Mallavarapu, G. R. 2011. Chemical diversity in curry leaf (*Murraya koenigii*) essential oils. Food Chem. 126: 989-994.

Shivankar, V. J., Rao, C. N., and Singh, S. 2000. Studies on citrus psylla, *Diaphorina citri*: A review. Agric. Rev. 29: 199-204.

Soroker, V., Talebaev, S., Harari, A. R. and Wesley S. D. 2004. The role of chemical cues in host and mate location in the pear psylla *Cacopsylla bidens* (Homoptera: Psyllidae). J. Insect Behav. 17: 613-626.

Wenninger, E. J., Stelinski, L. L., and Hall, D. G. 2009. Roles of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to four different host plants. Env. Ent. 38: 225-234.

Westbrook, C. J., Hall D. G., Stover, E., and Duan Y. P. 2011. Colonization of *Citrus* and *Citrus*-related germplasm by *Diaphorina citri* (Hemiptera: Psyllidae). HortScience 46: 997-1105.