



Response of *Diaphorina citri* (Hemiptera: Liviidae) to volatiles characteristic of preferred citrus hosts

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Abstract

The Asian citrus psyllid, *Diaphorina citri*, is currently the most important pest of citrus worldwide because it vectors ‘*Candidatus Liberibacter*’ spp., the etiological agents of lethal Huanglongbing (HLB). Reduction of *D. citri* populations is a key component of HLB management. Identifying potential *D. citri* attractants, such as volatiles, could be useful for behavioral management of this insect. This could, for instance, enhance catches of adults on monitoring traps. The overall aim of this study was to investigate the response of *D. citri* to volatiles characteristic of preferred citrus hosts. An ancillary objective was to formulate a synthetic blend of volatiles based on preferred host odors that could enhance monitoring traps. Volatile collections of two preferred citrus species, Duncan grapefruit (*Citrus paradise*) and sweet orange (*Citrus sinensis*), were performed in aeration chambers and analyzed by gas chromatography-mass spectrometry. Based on the grapefruit volatile profile, a synthetic blend consisting in D-limonene (0.62 mg), methyl *N*-methylantranilate (0.19 mg), β -ocimene (0.11 mg), β -elemene (0.02 mg), and β -caryophyllene (0.02 mg) was prepared in 9.9 mL of dichloromethane and evaluated for attractiveness, as well as the individual compounds, and compared with a positive control of natural volatiles collected from *Citrus sinensis*. Behavior of *D. citri* adults was evaluated in two-choice behavioral assays comparing response to lures with different combinations and dosages of the volatiles deployed on yellow sticky traps. Individually, neither D-limonene nor methyl *N*-methylantranilate affected behavior of *D. citri*. However, these volatiles increased catches of *D. citri*, as compared with solvent control, when presented as components of the synthetic grapefruit blend, as did the natural citrus odor positive control. Importantly, catch of *D. citri* appeared to vary with the release rate of the identified grapefruit volatile blend, which was attractive at a 0.1 mg loading dosage, but repellent at a dosage only 1 log step higher.

Keywords Asian citrus psyllid · Citrus greening · Citrus volatiles · Semiochemicals attractant · Host location

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Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the most serious pest of citrus worldwide, due primarily to its role as a vector of “*Candidatus Liberibacter asiaticus*” (Las) (Grafton-Cardwell et al. 2013). The bacterium causes the highly destructive citrus disease, citrus greening, also called Huanglongbing (HLB), which has no cure (Bové 2006). Worldwide, control of *D. citri* has been one of the three critical components of HLB management, which also includes planting pathogen-free nursery stock and removing the inoculum by destroying infected trees (Bové 2006). Due to the difficulty in detecting early Las infection in trees and the rapid spread of HLB, management programs in America have focused on vector control (Grafton-Cardwell et al. 2013).

The management strategy for *D. citri* has been based mainly on the use of insecticides, leading to an increase of annual insecticide applications (Boina and Bloomquist 2015). This strategy has many associated drawbacks, including *D. citri* insecticide resistance, negative consequences for beneficial fauna and for the environment, and destabilizing changes in the biological balance of agro-ecosystems (Beloti et al. 2017; Chen et al. 2017; Qureshi and Stansly 2010). Thus, the development of effective alternatives to control *D. citri* is still a matter of critical importance. Much research effort is currently aimed at finding potential attractants and repellents for *D. citri*.

Colored sticky traps are widely used for detection and population monitoring of *D. citri*, but their effectiveness varies with population density. For example, Miranda et al. (2018) showed that, taking into account costs of material and labor, sticky traps were an effective method for detecting *D. citri* under low pest densities; whereas, the opposite results were reported by Godfrey et al. (2013) under similar pest densities, when comparing multiple methods of pest monitoring. Hence, effective lures to increase *D. citri* captures are sorely needed. Using host-plant volatiles could increase the attractiveness of yellow sticky traps for monitoring the activity of *D. citri* adults and facilitate development of *attract-and-kill* technologies (Aksenov et al. 2014; Coutinho-Abreu et al. 2014; Yan et al. 2015). However, progress toward the development of these strategies for practical use has been slow with variable results (Grafton-Cardwell et al. 2013).

Host finding behavior in psyllids is complex and comprises several sensory modalities (Patt and Setañou 2010). Many 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 *D. citri* (Martini et al. 2014a; Patt et al. 2011; Patt and Setañou 2010; Stockton et al. 2016; Wenninger et al. 2009). The role of odor cues for *D. citri* orientation to host plants has been extensively investigated and reviewed (Mann et al. 2011; Patt and Setañou 2010; Setañou et al. 2012; Wenninger et al. 2009). While host-plant odor itself may not function as an orientation cue, it may enhance psyllid responsiveness to visual cues (Wenninger et al. 2009). *D. citri* responsiveness to the odor emitted by flushing shoots may depend on several different odor characteristics, such as the qualitative and quantitative composition of the volatiles, as well as, their release rate (Aksenov et al. 2014; Patt and Setañou 2010). However extensively researched, most of these studies have been carried out under controlled conditions, and *D. citri* behavior is still not understood completely.

The overall aim of this study was to investigate the response of *D. citri* to odors of preferred hosts, under less artificial conditions, for potential contribution to behavioral management of this insect. Volatile organic compounds (VOCs) emitted by two preferred citrus genotypes, sweet

orange (*Citrus sinensis*) and Duncan grapefruit (*Citrus paradisi*) (Alves et al. 2014; Liu and Tsai 2000; Setañou et al. 2008), were characterized by gas chromatography-mass spectrometry. Based on the volatile profile of Duncan grapefruit, synthetic volatiles were evaluated as a blend and compared with its major individual components comprising the blend (D-limonene and methyl *N*-methylantranilate). Volatiles were evaluated as lures deployed with yellow sticky traps in two-choice cage experiments. We identified a synthetic blend of volatiles that increased catch of *D. citri* compared to solvent control. In addition, our investigation revealed new insights regarding the influence of volatile host odors on *D. citri* host preference behavior.

Materials and methods

Host plants

VOCs were collected from Las-free, 8–10 month-old (20–25 cm tall) sweet orange (*Citrus sinensis*) and Duncan grapefruit (*Citrus paradisi*) potted seedlings. These were maintained in a greenhouse at the Estación Experimental INIA Salto Grande, Instituto Nacional de Investigación Agropecuaria, Salto, Uruguay (−31.32°, −57.94°). Plants were maintained at 27–28 °C, with 60–65% relative humidity and a 14:10 (L:D) photoperiod without exposure to insecticides.

Insects

Diaphorina citri used in the cage experiments were obtained from weekly field collections and maintained on orange jasmine, *Murraya paniculata* (L.) Jack., in a greenhouse at 27 ± 1 °C, 63 ± 2% RH and under a L14:D10 photoperiod at the University of Florida Citrus Research and Education Center in Lake Alfred, Florida. Illumination in the greenhouse was supplemented with linear fluorescent 54 W lights (F54W/T5/865/ECO, GE lighting, Nela Park, OH). The culture was established in 2000 from field populations collected in Polk Co., FL, USA (28.0°N, 81.9°W) before confirmation of Las in Florida.

Volatile collection and analysis

Leaf VOCs were obtained from potted undamaged seedlings enclosed in glass chambers (37.5 cm height, 24.0 cm diam.) sealed with Teflon lids. VOCs emitted were adsorbed during 48 h on 50 mg of Haysep-Q 80/100 mesh, using a stream of charcoal-filtered humidified air (300 mL/min). The flow was achieved pumping air to the system through a N035 At.18 bomb (KNF, Germany), and air suctioning, after air passed the adsorbent, through Personal Air Sampling Apex, Casella Cel. During the duration of VOC collection, plants

were kept at 22 ± 2 °C, $45 \pm 5\%$ RH and under a 14:10 h light : dark photoperiod. A total of 6 replicates (1 pot containing 2 seedlings comprised a replicate) and two blank controls were performed. Collections were made on two different collection dates. The VOCs were eluted with 1 mL bi-distilled hexane (J. K. Baker) and concentrated to 100 μ L under a stream of Nitrogen for gas chromatography coupled to mass spectrometry (GC–MS) analysis. GC–MS analyses were done using a QP-2010 Shimadzu GC–MS, equipped with a non-polar (AT-5 MS) column (Alltech, 30 m \times 0.25 mm, 0.25 μ m), and operated with a constant carrier (He) flow of 1 mL/min. The oven temperature was programmed as follows: the initial temperature was 40 °C (1 min), then increased to 280 °C at 5 °C/min, and held for 1 min at 280 °C. The injector temperature was 250 °C and the interphase temperature 280 °C. Injections (1 μ L) were in the splitless mode, and mass spectra were acquired from m/z 28 to 350 (70 eV, scan mode). For retention index calculations, a mixture of n-alkanes (100 ppm each, in hexane) was injected in the splitless mode immediately after the samples. Compounds were identified tentatively through comparison of their retention indexes and mass spectra with the ones reported in the database Adams and NIST (Adams 2007; NIST Mass Spec Data Center 2017), as well as comparison with retention indexes and mass spectra of synthetic standards injected in the same conditions. Differences in content of compounds were determined by comparison of relative areas.

Preparation of stimuli

A synthetic blend mimicking the volatile emissions of Duncan grapefruit was prepared (grapefruit blend). Because the Duncan grapefruit volatile profile was observed to be less complex than the one from sweet orange (see “Results” section), it was selected for preparation of a synthetic blend for behavioral analysis. The five compounds that constituted about 96% of the composition of the VOCs profile (relative percentages) were selected: D-limonene 62%, methyl *N*-methylantranilate (MNMA) 19%, β -ocimene 11%, β -elemene 2% and β -caryophyllene 2% (Table 2). A synthetic volatile lure treatment based on the profile of grapefruit was evaluated at two dosages, 0.1 (referred to as GB/10) and 1.0 mg (referred to as GB), released from polyethylene vials, described below, that were attached to yellow sticky traps. The attractiveness of D-limonene and MNMA alone was also evaluated. In all cases, a 100 μ L total of corresponding dilution was loaded into vials and dichloromethane (DCM) was used as solvent. Pure compounds were obtained from Sigma-Aldrich y TCI Chemicals America. Purity of compounds used were as follows: D-limonene 97%, MNMA > 95%, β -ocimene \geq 90% (mixture of isomers), β -elemene \geq 98% and β -caryophyllene \geq 80%.

Actual amounts of each compound loaded into release vials and all treatments evaluated are summarized in Table 1.

To create a positive control treatment for behavioral evaluation, VOCs were obtained from sweet orange over a 48-h collection period according to the methods described above. The volatile lure consisted of a solution obtained by eluting the adsorbent columns with 1 mL of DCM. Sweet orange was used for this purpose because the seedlings were available at the time when behavioral assays were being conducted. DCM alone was used as a solvent control.

Two-choices cage experiment

To allow air flow inside the experimental arena, white-mesh cages (90 \times 60 \times 55 cm) were used. At each opposite side of length of the cages, 8 \times 8 cm yellow sticky traps were placed, one containing 100 μ L of the stimulus solution (Table 1), and the other 100 μ L of DCM only. Volatiles were released from polyethylene vials (BEEM 1001 Capsules for Embedding, Size 00). The loading of 100 μ L on the vials was established according to the loading of stimulus applied in olfactometry experiments in the authors’ previous research (Aksenov et al. 2014; Martini et al. 2014b). Forty adult *D. citri* were released in the middle of each cage and 24 h later, the number of adults captured on each trap was recorded. Cages were distributed randomly in a glass greenhouse (8.0 \times 9.60 \times 6.30 m approx.) used for plant production, mostly citrus seedlings culture, and one treatment was assigned to each cage. A randomized complete block design was used, where each treatment was evaluated once per date, and the whole experiment was repeated 10 times on consecutive days with each date considered as a block. For each replicate, treatments were randomly assigned to different positions inside the cages to avoid positional bias. All tests were conducted at 35 ± 6 °C, $69 \pm 21\%$ RH, and a 12:12 L:D photoperiod.

Table 1 Amounts of each compound (mg) loaded into polyethelene vial dispenser attached to yellow traps in various choice experiments

	mg applied (in 100 μ L)			
	GB	GB/10	D-Limonene	MNMA
D-Limonene	0.62	0.062	0.062	– ^a
Methyl <i>N</i> -methylantranilate	0.19	0.019	–	0.019
β -Ocimene	0.11	0.011	–	–
β -Elemene	0.02	0.002	–	–
β -Caryophyllene	0.02	0.002	–	–

The control trap was treated with 100 μ L of dichloromethane in each experiment. GB indicates grapefruit blend at 1.0 mg dosage. GB/10 indicates grapefruit blend at 0.1 mg dosage. MNMA is methyl *N*-methylantranilate

^aDoesn’t apply, unit compound stimuli

Table 2 Chemical composition of the VOCs from sweet orange and Duncan grapefruit

Retention time	Compound	Compound family	Retention index		Duncan grapefruit		Sweet orange	
			Rep. ^a	Calc. ^b	Media ± SE	Min–max	Media ± SE	Min–max
9.35	Sabinene	MT	969	973	1.1 ± 0.6	0.0–2.5	2.0 ± 0.4	0.6–3.5
9.45	β-Pinene	MT	974	977	0.3 ± 0.1	0.0–0.6	0.16 ± 0.09	0.00–0.58
9.85	β-Myrcene	MT	988	992	– ^c	–	1.0 ± 0.1	0.8–1.5
9.93	Mesitylene	Aromatic	994	995	–	–	0.2 ± 0.1	0.0–0.8
10.44	δ-3-Carene	MT	1008	1012	–	–	10 ± 9	0–57
10.87	p-Cymene	MT	1020	1025	0.5 ± 0.3	0.0–1.6	–	–
11.07	Limonene	MT	1024	1031	67 ± 6	51–89	41 ± 10	0–65
11.25	(E)-β-ocimene	MT	1044	1039	7 ± 5	0–24	0.6 ± 0.5	0.1–3.0
11.57	Monoterpene hydrocarbon	MT	x ^d	1051	–	–	2.2 ± 0.8	0.6–5.8
11.89	γ-Terpinene	MT	1054	1061	–	–	1.1 ± 0.2	0.7–1.9
13.08	Linalool	MT oxig	1095	1101	–	–	0.6 ± 0.2	0.0–1.0
13.22	Nonanal	Aldehyde	1110	1106	–	–	0.3 ± 0.3	0.0–1.7
13.58	Unknown	Unknown compound	x	1118	0.6 ± 0.4	0.0–2.0	–	–
13.60	(E)-thujone	MT oxig	1112	1119	–	–	0.07 ± 0.07	0–0.4
14.25	trans-Limonene oxide	MT oxig	1137	1141	–	–	0.30 ± 0.06	0.11–0.48
15.80	α-Terpineol	MT oxig	1186	1193	–	–	0.03 ± 0.03	0.00–0.18
16.00	Dodecane	Hydrocarbon	1200	1200	–	–	0.4 ± 0.2	0.0–1.2
16.18	Decanal	Aldehyde	1201	1206	–	–	1.9 ± 0.6	0.1–3.8
16.62	cis-Carveol	MT oxig	1226	1223	–	–	0.05 ± 0.05	0.00–0.29
17.35	Carvone	MT oxig	1239	1248	–	–	0.18 ± 0.04	0.05–0.36
17.76	(2E)-decenal	Aldehyde	1260	1264	–	–	0.9 ± 0.3	0.2–2.1
18.54	Bornyl acetate	MT oxig	1287	1294	–	–	0.22 ± 0.07	0.00–0.46
18.78	Tridecane	Hydrocarbon	1300	1300	–	–	0.22 ± 0.06	0.08–0.41
19.90	δ-Elemene	ST	1335	1342	–	–	0.15 ± 0.07	0.00–0.39
20.20	Methylanthranilate	MA	1334	1349	–	–	0.4 ± 0.2	0.0–1.0
20.52	(2E)-undecenal	Aldehyde	1357	1366	–	–	0.6 ± 0.3	0.0–1.5
21.34	β-Elemene	ST	1389	1396	1.0 ± 0.6	0.0–2.5	0.09 ± 0.06	0.00–0.30
21.43	Alkane	Hydrocarbon	x	1416	–	–	0.2 ± 0.2	0.0–1.1
21.75	Methyl N-methylanthranilate	MA	1405	1416	21 ± 5	6–35	33 ± 5	18–48
22.11	β-Caryophyllene	ST	1417	1430	2.0 ± 0.6	0.6–3.9	1.6 ± 0.3	0.6–2.5
Compound family		Media ± SE	Min–max		Media ± SE	Min–max		
Monoterpene hydrocarbon (MT)		76 ± 5	58–89		58 ± 6	38–72		
Oxygenated monoterpenes (oxyg MT)		0 ± 0	0–0		1.5 ± 0.2	0.8–2		
Sesquiterpene hydrocarbons (ST)		3 ± 1	0.6–6.4		1.8 ± 0.4	0.6–3		
Methyl anthranilates (MA)		21 ± 5	6–36		33 ± 5	18–48		
Others (aldehyde, ketone, aromatic, hydrocarbons and unknown compounds)		0.6 ± 0.4	0–2		5 ± 1	2–9		

Data were obtained from the total ion chromatograms (TIC). Specific compounds are tentatively identified from comparison of their mass spectra with data on available databases (Adams 2007); as well as comparison with retention indexes and mass spectra of synthetic standards injected in the same conditions. Quantification was made based on normalized area of each TIC

^aReported in Adams (2007)

^bCalculated according to Adams (2007)

^cNot detected

^dSpecific compound couldn't be identified

Statistical analysis

The pooled number of psyllids caught on each trap during the 10 replicates of the choice tests were analyzed with Chi square tests for within-group comparisons. Beforehand, we carried out heterogeneity chi-squared tests to ensure that data from each replicate were homogenous (Zar 2009). In the case of non homogenous data (D-limonene), paired *t* tests with square root transformed data were performed. The counts of insects per trap, considering the total catches per replicate, were subjected to a generalized linear model (GLM) with binomial distribution and treatment means were compared using Tukey's test at an $\alpha=0.05$. Relative amounts of monoterpenes, sesquiterpenes and oxygenated monoterpenes (compound families) between sweet orange and Duncan grapefruit were compared by analysis of variance (ANOVA) followed by Tukey's test at an $\alpha=0.05$. Tests were run with R statistical software (0.99.892 version – © 2009–2016 RStudio, Inc.) (RStudioTeam 2015) and Infostat statistical software (Di Rienzo et al. 2011).

Results

Volatile collection and analysis

Profiles of leaf VOCs from the two genotypes exhibited quantitative and qualitative differences (Fig. 1; Table 2). There were 28 compounds in the profiles of sweet orange and 9 in Duncan grapefruit (Fig. 1; Table 2). VOCs from both genotypes were primary monoterpene hydrocarbons (58 ± 6 and $76 \pm 5\%$ in sweet orange and Duncan grapefruit,

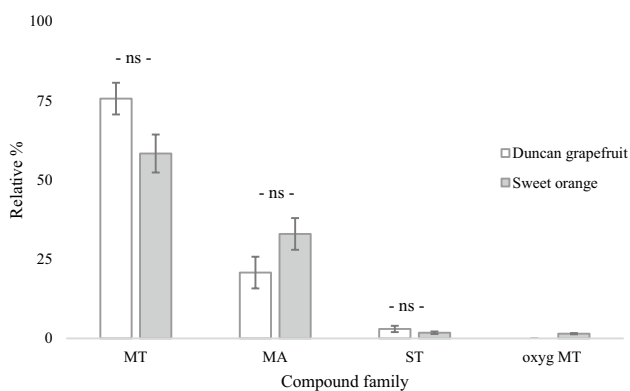


Fig. 1 Relative percentage of compounds grouped by families. Results are shown as media \pm standard error. *MT* monoterpene hydrocarbons, *MA* methyl anthranilates, *ST* sesquiterpene hydrocarbons, *oxyg MT* oxygenated monoterpenes. The remaining percentage is accounted by other compounds belonging to other chemical classes (see Table 1). *ns* not significant differences between both genotypes (ANOVA, $p > 0.05$ in all cases; oxyg MT were not detected in Duncan grapefruit)

respectively). Among these monoterpenes, limonene was the most abundant one in both species ($41 \pm 10\%$ and $67 \pm 6\%$, respectively; Table 2). Furthermore, high relative amounts of methyl *N*-methylantranilate were found in both genotypes ($33 \pm 5\%$ in sweet orange and $21 \pm 5\%$ in Duncan grapefruit). Oxygenated monoterpenes (present only in sweet orange, $1.5 \pm 0.4\%$) and sesquiterpene hydrocarbons were also detected in lower proportions ($1.8 \pm 0.4\%$ in sweet orange, $3 \pm 1\%$ in Duncan grapefruit). No significant differences in relative amounts of the above-mentioned compound families were found between genotypes (ANOVA, Tukey test $p > 0.05$, Fig. 1). Miscellaneous compounds found in lower abundance (aldehyde, ketone, aromatic, hydrocarbons, and unknown compounds) were more prevalent in sweet orange ($5 \pm 1\%$) than in Duncan grapefruit ($0.6 \pm 0.4\%$).

Cage experiments

Significantly more *D. citri* were captured on traps baited with 0.1 mg of the synthetic grapefruit blend than on traps with the solvent control ($\chi^2 = 10.45$, d.f. = 1, $p = 0.001$) (Fig. 2). Catch of *D. citri* were increased by two-fold on traps baited with this dosage of the synthetic grapefruit blend as compared with the control. Similarly, significantly more *D. citri* were captured on traps baited with natural VOCs from sweet orange than on the solvent control ($\chi^2 = 5.76$, d.f. = 1, $p = 0.016$); representing a 1.5-fold increase in capture relative to the control (Fig. 2). D-Limonene and MNMA stimuli did not affect the catches of *D. citri* relative to solvent baited

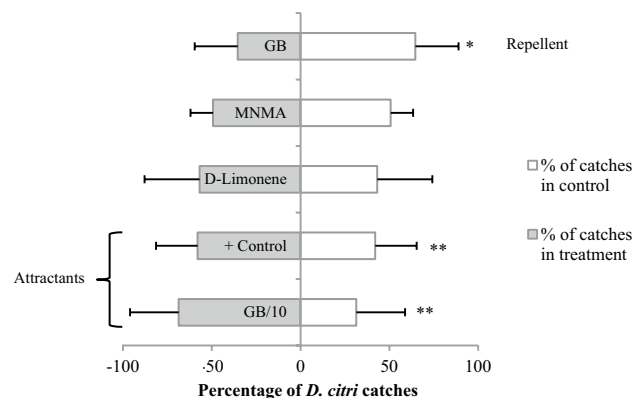


Fig. 2 *Diaphorina citri* catches (%) in choice experiments. White bars indicate the percentage of *D. citri* caught on control traps. Gray bars indicate percentage of *D. citri* caught on traps baited with the indicated stimulus. Lines outside the bars show the standard deviation. Asterisks (*) outside bars indicate differences between treatment and control (χ^2 test, $*p < 0.05$, $**p < 0.01$). GB indicates grapefruit blend at 1.0 mg dosage. GB/10 indicates grapefruit blend at 0.1 mg dosage. MNMA is methyl *N*-methyl anthranilate (mg applied are detailed in Table 1). The positive control is a natural volatile mixture collected from *Citrus sinensis*

control traps (paired *t* test, $p=0.472$ and: $\chi^2=0.94$, d.f. = 1, $p=0.334$, respectively).

Catch of *D. citri* was significantly lower on traps baited with the synthetic grapefruit blend at the 1.0 mg dosage as compared with solvent baited controls ($\chi^2=10.33$, d.f. = 1, $p=0.001$), indicating this volatile blend was repellent at the higher dosage. Captures of *D. citri* on traps baited with the synthetic grapefruit blend at the 0.1 mg loading dosage and traps baited with the natural sweet orange odor (positive control) were not statistically different from one another, but both were higher than that captured on the GB (Fig. 3, GLM, $p<0.05$).

Discussion

The profile of citrus VOCs has been investigated extensively and reported to contain a mixture of monoterpene and sesquiterpene hydrocarbons and oxygenated monoterpenes (Beloti et al. 2017; Killiny and Jones 2017; Patt and Setaïou 2010; Robbins et al. 2012). Our results were consistent with previous reports using similar in vivo methodologies of VOCs collection (Patt and Setaïou 2010; Robbins et al. 2012) and indicated high proportions of monoterpene hydrocarbons (Fig. 1) in both genotypes. Our results also indicated that limonene was the most abundant compound in both profiles (Table 2). This compound has also been described as a predominant citrus leaf volatile by other authors (Killiny and Jones 2017; Mann et al. 2012; Robbins et al. 2012). An important difference in our investigation was the relatively high amount of methyl

N-methylantranilate found in both species as compared with previous studies. This compound has been reported in essential oils from sour orange (*Citrus aurantium* L.) (Aragão da Hora Almeida et al. 2015) and leaf volatiles analyzed through solvent extraction (Gancel et al. 2003) but not, to our knowledge, as a citrus leaf volatile from Duncan grapefruit or sweet orange obtained from in vivo collections (Beloti et al. 2017; Killiny and Jones 2017; Patt and Setaïou 2010; Robbins et al. 2012). Both Duncan grapefruit and sweet orange VOCs profiles reported here were similar with respect to the families of compounds characterizing both profiles (Fig. 1). However, differences were found with respect to complexity of each profile and certain specific compounds that distinguished the two profiles. Despite these differences, the synthetic blend mimicking Duncan grapefruit profile (GB/10) and the natural sweet orange VOCs showed similar attractiveness to *D. citri* in cage experiments (Fig. 3). Indeed in PCA analysis of VOCs from multiple genotypes, these two species have been grouped together (Hijaz et al. 2016).

When evaluated at the lower 0.1 mg dosage, the synthetic volatile blend from Duncan grapefruit, GB/10, was roughly equivalent to a natural volatile collection from sweet orange (the positive control, Fig. 3) with regard to enhancing catch of *D. citri* on yellow monitoring traps. However, there was substantial variance in the results obtained, which can be visualized in the dispersion of the data represented in the boxplots in Fig. 3. The variation in *D. citri* response to the synthetic volatile blend as compared with the natural sweet orange odor positive control suggests possible hypotheses for future investigation.

First, the VOC profiles of the host-plant odors are characterized by likely important complexity, which may need to be retained in a synthetic volatile employed as an effective lure for *D. citri* monitoring. Sweet orange VOCs (the positive control), which maintain the complexity and the proportions of odors that *D. citri* encounters in its natural environment, showed a more consistent effect (never less than 50% of insects on treated trap) than the low dosage synthetic grapefruit blend (which has a minimum of 20% of catches on treated trap). Also, it is interesting that the VOC profiles of the two preferred genotypes investigated here (Alves et al. 2014; Liu and Tsai 2000; Setaïou et al. 2008) were characterized by relatively high proportions of *D*-limonene and MNMA and yet, at the evaluated doses, neither of the individual compounds increased attraction of *D. citri* to yellow sticky traps. Perhaps, this absence of effect on the behavioral preference of *D. citri* is indicative of the requirement of integrating a complex blend to elicit a behavioral response to host-plant odor from *D. citri*. It is also possible that some of the volatile and non-volatile compounds characterizing host-plant chemistry of *D. citri* interact additively or synergistically when perceived as blends [reviewed by Bruce and

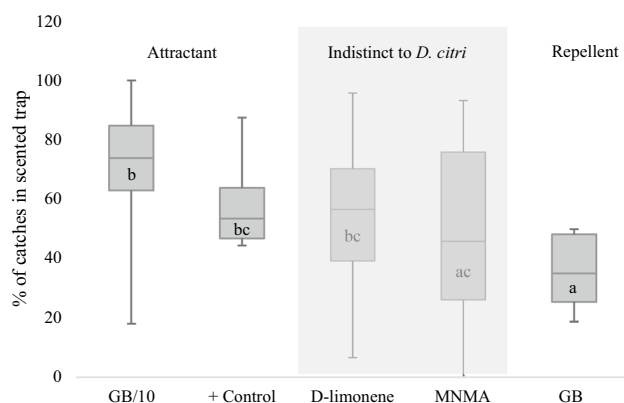


Fig. 3 Comparison of *D. citri* response to synthetic volatile odors. Results are shown as percentage of insects caught after 24 h on baited traps. Boxes show the 1st and 3rd quartiles; vertical lines indicate maximum and minimum. Medians with the same letter indicate no significant differences (GLM, Tukey–Kramer $p>0.05$). GB indicates grapefruit blend at 1.0 mg dosage. GB/10 indicates grapefruit blend at 0.1 mg dosage. The positive control is a natural volatile mixture collected from *Citrus sinensis*. Gray area indicates response to cue(s) causing no effect on *D. citri* behavior (χ^2 test, $p>0.05$)

Pickett (2011)] by foraging psyllids as has been reported previously with *D. citri* (Patt and Setañou 2010). Collectively, our current data and results from previous work (Patt and Setañou 2010) strongly suggest that *D. citri* response toward host volatiles is mediated by odor blends rather than individual components comprising those blends.

In addition to blend complexity, the release rate of the identified grapefruit volatile blend appeared to significantly influence catch of *D. citri* on monitoring traps. The effect of the synthetic grapefruit volatile blend on captures of *D. citri* exhibited a typical biphasic response attracting psyllids at a lower (presumably more natural) release rate of odors (0.1 mg loading dosage), but repelling them at a higher dosage that was likely above a threshold for attraction (1.0 mg loading dosage) (Fig. 2). Similar findings with *D. citri* have been described previously. For example, Mann et al. (2012) found differential response of *D. citri* to varying release rates of D-limonene in olfactometer tests with attraction increasing in a dosage dependent manner. In our experiment, although D-limonene caught 1.4 times more *D. citri* than the respective untreated trap (Fig. 2), the variability in response among replicates prevented resolution of a statistical difference from the control. In addition, Martini et al. (2016) demonstrated that pre-exposing *D. citri* to unnaturally high concentrations of certain plant volatiles alters their subsequent ability of to select hosts based on odor preference. Finally, β -caryophyllene has been recently reported as a *D. citri* repellent at high doses (Alquézar et al. 2017); this could explain the repellent activity of the high dosage (1 mg) synthetic grapefruit blend, which included 2% of β -caryophyllene. In general terms, repellency of known insect attractants at abnormally high release doses is a well-known phenomenon (Dethier 1947). Therefore, optimization of the release rate of a semiochemical attractant lure for *D. citri* will likely be of significant importance and deserves further attention.

Our results reflect the complexity of *D. citri* behavior and in particular, the difficulty of manipulating psyllid behavior with volatile semiochemicals. The poor and variable response of *D. citri* to odors has been commonly observed in olfactometry (Alquézar et al. 2017), semi-field (Patt and Setañou 2010) and field trials of scented traps with both candidate attractants (Godfrey et al. 2013) and repellents (Kuhns et al. 2016). The weak response to natural VOCs has also been described for other psyllid species (Steinbauer 2016). In fact, to our knowledge, no efficient practical applications of *D. citri* chemical ecology research have been incorporated to management strategies yet, despite significant research effort (Grafton-Cardwell et al. 2013; Yan et al. 2015). The relative simplicity of *D. citri* antennae (Onagbola et al. 2009), may be suggestive that this species relies on other senses rather than olfaction when selecting host plants for nutrition, mate finding, and oviposition. Indeed,

visual cues, which may be integrated with semiochemical cues for *D. citri* management, are known to strongly affect orientation behavior of *D. citri* (Patt et al. 2011; Stockton et al. 2016). Recent research on the psyllid species *Cacopsylla pruni*, proved that sap composition is a crucial factor explaining host-plant alternation of this insect (Gallinger and Gross 2018). This is another example of the importance of non-volatile plant compounds on host acceptance of psyllids. In this respect, the combined use of both visual and chemical attractants and repellents, and in the context of a push and pull strategy where a trap crop may provide a necessarily complete set of host selection cues, may be necessary for behavioral modification of this species. In fact, current research efforts in *D. citri* chemical ecology are trending toward these kinds of strategies, where release of selected behavior modifying semiochemicals for *D. citri* is up-regulated from natural plants (Alquézar et al. 2017).

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