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Guava leaf volatiles and dimethyl disulphide inhibit response of *Diaphorina citri* Kuwayama to host plant volatiles

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Abstract

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, vectors the causal pathogen of huanglongbing (HLB), which is likely the most important disease affecting worldwide citrus production. Interplanting citrus with guava, *Psidium guajava* L., was reported to reduce *D. citri* populations and incidence of HLB. We describe a series of investigations on the response of *D. citri* to citrus volatiles with and without guava leaf volatiles and to synthetic dimethyl disulphide (DMDS), in laboratory olfactometers and in the field. Volatiles from guava leaves significantly inhibited attraction of *D. citri* to normally attractive host-plant (citrus) volatiles. A similar level of inhibition was recorded when synthetic DMDS was co-released with volatiles from citrus leaves. In addition, the volatile mixture emanating from a combination of intact citrus and intact guava leaves induced a knock-down effect on adult *D. citri*. Compounds similar to DMDS including dipropyl disulphide, ethyl-1-propyl disulphide, and diethyl disulphide did not affect the behavioural response of *D. citri* to attractive citrus host plant volatiles. Head-space volatile analyses were conducted to compare sulphur volatile profiles of citrus and guava, used in our behavioural assays, with a gas chromatography-pulsed flame photometric detector. DMDS, produced by wounded guava in our olfactometer assays, was not produced by similarly wounded citrus. The airborne concentration of DMDS that induced the behavioural effect in the 4-choice olfactometer was 107 pg/ml. In a small plot field experiment, populations of *D. citri* were significantly reduced by deployment of synthetic DMDS from polyethylene vials compared with untreated control plots. Our results verify that guava leaf volatiles inhibit the response of *D. citri* to citrus host plant volatiles and suggest that the induced compound, DMDS, may be partially responsible for this effect. Also, we show that field deployment of DMDS reduces densities of *D. citri* and thus may have potential as a novel control strategy.

Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), vectors bacteria in the genus *Liberibacter*, which are the causal agents of huanglongbing (HLB) (Tsai et al. 1988; Su and Huang 1990). HLB causes rapid tree decline, fruit loss, and eventual tree death (Capoor 1963; Roistacher 1996; Bové 2006). Since its introduction into Florida in 1998 (Halbert and Manjunath 2004), *D. citri* has

spread to all citrus-producing regions in the state becoming a major threat to US citrus production (Michaud 2001; Michaud and Olsen 2004).

Efforts to manage the *D. citri*-HLB complex in Florida include preventing the spread of *Liberibacter* through certification of budwood that has been maintained in certified psyllid-free environments (Halbert and Manjunath 2004). A complex of syrphid (Aubert 1987; Michaud 2002) and coccinellid (Michaud 2002, 2004) predators and a eulophid ectoparasitoid, *Tamarixia radiata* (Waterston) (Tang 1989; McFarland and Hoy 2001), are known effective biological control agents of *D. citri*. However, these natural enemies do not impact *D. citri* populations sufficiently to reduce spread of HLB in Florida (Halbert and Manjunath 2004). Koizumi *et al.* (1993) suggested the need for development of HLB-resistant citrus cultivars; however, this has not been accomplished (Halbert and Manjunath 2004). In fact, all major commercial sweet oranges, mandarins, and tangelos are highly susceptible to HLB infection. Integrating cultural control of removing infected trees and replanting with clean nursery stock (Buitendag and von Broembsen 1993) with the use of insecticides (Childers and Rogers 2005) has also not been effective in preventing HLB spread in Florida. In fact, the toxic effect of insecticides on non-target biological control agents has induced outbreaks of secondary pests.

Interplanting guava, *Psidium guajava* L., with citrus has been reported to reduce populations of *D. citri* and this has been attributed to a repellent effect of guava volatiles (Beattie et al. 2006; Hall et al. 2008; Zaka et al. 2010). It has been suggested that certain toxic volatile metabolites produced by guava may repel psyllids or otherwise affect host plant finding (Hall et al. 2008; Zaka et al. 2010). Recently, we discovered that the sulphur compound, dimethyl disulphide (DMDS), was produced by guava in substantial quantities in response to mechanical injury to leaves (Rouseff et al. 2008). However, citrus host plants of *D. citri* did not produce this compound even after mechanical wounding (Rouseff et al. 2008). Balandrin *et al.* (1988) found a range of sulphur compounds including di-*n*-propyl disulphide in neem seeds [*Azadirachta indica* Adr. Juss. (Meliaceae)]. This compound is larvicidal to the yellow fever mosquito, *Aedes egypti* (L.) (Diptera: Culicidae) and to the noctuids *Heliothis virescens* (Fab.) and *Helicoverpa zea* (Boddie) (Balandrin et al.1988). Sulphur compounds from common garlic, *Allium sativum* L., are also known insect repellents and insecticides (Huang et al. 2000). Since DMDS is the major sulphur compound produced by wounded guava leaves, we hypothesized that it may be to some degree responsible for guava's effect on the behaviour of *D. citri*.

In this series of experiments, we investigated the effect of guava leaf volatiles on the behavioural response of *D. citri* to citrus volatiles. Our objectives were to compare the effect of authentic guava leaf volatiles on *D. citri* behaviour with that of synthetic DMDS, a known guava secondary metabolite (Rouseff et al. 2008) and insect repellent/neurotoxin (Dugravot et al. 2002, 2003). In addition, a small plot field investigation was conducted to determine the effect of DMDS on population densities of *D. citri*. The overall goal of this research was to determine whether a synthetic repellent could be useful for future management of *D. citri*.

Materials and Methods

Insects

Adult *D. citri* of mixed sex were drawn from a continuously reared culture at the University of Florida Citrus Research and Education Center (Lake Alfred, USA) and established in 2000 from field populations in Polk Co., FL, USA (28.0'N, 81.9'W) prior to the discovery of HLB in FL. This culture is maintained on sour orange (*Citrus aurantium* L.) and 'Hamlin' orange [*C. sinensis* (L.)] seedlings at 27 ± 1 °C, $63 \pm 2\%$ RH, and a L14:D10 photoperiod. Freshly emerged adult psyllids were first placed on citrus seedlings in Plexiglas cages for up to 7 days for sexual maturation prior to use in experiments.

Leaf samples

Husbandry methods for the 'white' guava (*Psidium guajava* L.; Myrtaceae) and 'Hamlin' citrus (*Citrus sinensis* L.; Rutaceae) plants used in these investigations have been described previously (Rouseff et al. 2008). 'Hamlin' citrus was selected for analysis because it is one of the most highly cultivated citrus varieties in Florida. Secondary plant metabolites are typically not evenly distributed within plants (Loomis and Croteau 1980). In order to maximize the amount of static volatile metabolites for analyses, we used fresh leaf flush [immature leaves at the growing shoots (Hall and Albrigo 2007)], which are known to contain a higher proportion of plant metabolites (Hrutfiord et al. 1974) compared with older leaves or other plant parts.

Response of *D. citri* to citrus with guava volatiles or DMDS

Behavioural responses of *D. citri* to citrus volatiles with or without guava or DMDS volatiles were quantified using a 4-choice olfactometer (Analytical Research Systems, Gainesville, FL, USA) based on the design of Pettersson (1970), Vet *et al.* (1983), and Kalule and Wright (2004). The olfactometer consists of a 5 cm × 30 cm × 30 cm Teflon stage on four 2.5 cm × 15 cm legs with extending arms on each of the four sides of the stage. Charcoal-purified and humidified air was drawn through these arms via a vacuum pump that created four potential odour fields. Air pulled through the olfactometer arms was then evacuated through a central orifice on the floor of the stage. The orifices of the olfactometer were connected through Teflon-lined glass tube connectors to four pumps on an air delivery system equipped with a vacuum pump (Analytical Research Systems), which suctioned air out of the olfactometer through a central orifice (Mann et al. 2010). A constant airflow of 0.1 l/min was maintained through each of the four orifices and a 0.5 l/min suction flow was maintained to vacuum the odour mixture from the olfactometer. Two fluorescent lights (~250 lux) were positioned centrally above the olfactometer, which were housed within a 76 cm × 81 cm × 86 cm white fibre board box for uniform light diffusion. A second 25 cm × 30 cm × 30 cm box, whose inside walls and roof were lined with black cloth, was placed directly over the olfactometer to completely shield the stage of the olfactometer from light, but not the traps and the extending arms. All experiments were conducted at 25 ± 1 °C and 60 ± 5% RH.

In order to exclude the possibility of positional bias, the behavioural response of adult *D. citri* to clean laboratory air was first investigated using four blank arms of the olfactometer as a negative control. Thereafter, the response of *D. citri* was measured to citrus leaf volatiles with and without volatiles from guava leaves or synthetic DMDS. For each treatment, only two of the four possible arms received volatile treatments. The treatments compared were: (i) intact citrus alone; (ii) intact citrus + DMDS; (iii) crushed citrus + DMDS; (iv) DMDS alone; (v) crushed guava + crushed citrus; (vi) intact guava + intact citrus; (vii) crushed guava + intact guava, and (viii) crushed citrus + intact citrus. In certain treatments, leaves were gently crushed (using a clean glass rod) to simulate plant damage. All samples (3.5 g leaf flush/sample) were wrapped in 4.4 × 8.4 cm disposable tissues (Kimwipe, Kimberly-Clark®, Ontario, Canada) and placed in 2.5 cm × 12.5 cm extending glass tubes of the olfactometer. Ten *D. citri* were assayed per replicate and each experiment was replicated at least 15 times (≥150 *D. citri* assayed per treatment concentration). The number of psyllids found in the extending arms of the olfactometer as well as the number not moving from the release point 1.5 h following psyllid release was recorded.

In treatments investigating synthetic DMDS (Aldrich Chemical Company, Milwaukee, WI, >96% pure), 100 μl of a 4.3 μg/μl (w/v) solution of DMDS in mineral oil (Aldrich Chemical Company) was pipetted onto a 1 × 1 cm braided piece of Richmond cotton wick (Petty John Packaging, Inc., Concord, NC) to slow release rate (Arthur 1996, Dugravot et al. 2002). The mineral oil was found not to affect behaviour of *D. citri* in our bioassays (Mann et al. 2010). The DMDS was mixed with mineral oil to reduce release during bioassays, given its high volatility (Dugravot et al. 2004). This dosage was selected based on a preliminary investigation that showed it to be the lowest dosage to affect attraction of *D. citri* to citrus volatiles, compared with lower dosages tested on a log scale (data not shown).

After each run, the olfactometer as well as the glass tubes were first washed in soapy water and rinsed with distilled water. The glass tubes were then rinsed with acetone and the olfactometer stage (made of Plexiglass and Teflon) was cleaned with absolute ethanol. Thereafter, the olfactometer was air dried.

A Y-tube olfactometer study was conducted to further investigate the effect of DMDS on response of *D. citri*. The Y-tube (Analytical Research Systems, Gainesville, FL, USA) consisted of a central stem (13.5 cm long, 2.4 cm o.d.) with two lateral arms (5.8 cm long, 2.4 cm o.d.). The lateral arms were connected to extending glass tubes (14.5 cm long, 1.9 cm o.d.) with inlayed sieves (5.3 cm away from connection) to prevent insect escape and serve as an end point of the lateral arms. Charcoal-filtered laboratory air was passed from an air pump into each of the extending arms of the olfactometer at a rate of 100 ml/min. The Y-tube was suspended vertically on a clear plexiglass plate and placed in the white fibreboard box, described above, for uniform light diffusion and to minimize visual distraction of the adult *D. citri*. Two seperate Y-tube experiments were conducted to investigate the effect of DMDS on response of *D. citri* to citrus volatiles. In the first experiment, a mixture of volatiles from 100 μl of the DMDS solution in mineral oil described above and 3.5 g of

citrus flush were simultaneously presented to the adult psyllids from both extending arms of the Y-tube. The negative control for this experiment consisted of 3.5 g of citrus flush alone (without DMDS) in each arm of the Y-tube. In experiment 2, adult *D. citri* were presented with ~3.5 g of citrus flush or the DMDS solution, described above, in one extending arm of the olfactometer vs. clean laboratory air in the other arm. In experiment 1, we quantified the number of *D. citri* that did not move from the release point compared with the number contacting the source of volatiles. In the second experiment, we compared the number of *D. citri* choosing arms containing a source of volatiles vs. arms containing clean air. The first choice of each *D. citri* was recorded as the arm in which the psyllid first entered 1 cm into the arm. The tested adult psyllids were released individually from the base of the olfactometer stem and given 300 s to exhibit a behavioural response. The arms of the olfactometer were rotated after three adult psyllids were tested and the entire system was cleaned after six psyllids had been tested. For each experiment, at least thirty adult *D. citri* (at least 15 per sex) were tested per treatment.

Response of *D. citri* to citrus and DMDS-related volatiles in a Y-tube olfactometer

A second Y-tube study was conducted according to the methods described for Y-tube experiment 2, described above, to determine the response of *D. citri* to other sulphide compounds with boiling points and molecular weights similar to DMDS. The hypothesis tested was that the activity of DMDS on *D. citri* is unique to this compound as opposed to occurring during exposure to disulphide compounds in general. For each experiment, one arm of the Y-tube received clean air, whilst the other received a volatile treatment. The treatments compared against clean air were: (i) 3.5 g of citrus flush alone, or the same amount of citrus leaf material with either: (ii) DMDS; (iii) dipropyl disulphide; (iv) ethyl-1-propyls disulphide, or (v) diethyl disulphide. All chemicals were obtained from the Aldrich Chemical Company (Milwaukee, WI) and were >95% pure. Each sulphide chemical was released from mineral oil at the rate described for DMDS above. Each volatile treatment vs. clean air combination was presented to male *D. citri* on each day of testing until 40 replicates were accrued per combination. For each replicate, a single psyllid was assayed until 20 males and 20 females were tested per treatment. All other methods were exactly as described above.

Analysis of volatiles from citrus and guava leaves

We collected head-space volatiles from leaf flush of 'white' guava or 'Hamlin' orange used in our behavioural assays, by static solid phase micro extraction (SPME) technique similar to that described in Rouseff *et al.* (2008). Leaf flush from guava and citrus were harvested and weighed on a Mettler® AE 160 balance (Greifensee, Switzerland). Approximately 3.5 g of guava or citrus leaves was weighed into 40 mL septum-sealed glass vials, which were allowed to equilibrate at 23 ± 1 °C for 30 min. Accumulated static head-space volatiles were collected from the glass vials at 0, 10, 30 and 60 min post exposure. Static head-space volatiles were collected using a 75 μm Carboxen-polydimethylsiloxane (PDMS) Stable Flex® SPME fibre (Supelco, Bellefonte, PA). At least three replicates of each static volatile sample were analysed.

Sulphur compounds were analysed using a pulsed flame photometric detector (PFPD) (Model 5380; OI Analytical Co., College Station, TX, USA) set up in the sulphur mode coupled to a HP-5890 Series II GC. The PFPD specifically detects presence of sulphur and carbon in volatile samples. The GC was equipped with a 30 m × 0.32 mm. i.d. × 0.5 μm ZB-5 (Zebron ZB-5; Phenomenex, Torrance, CA, USA) capillary column and programmed from 40 to 265°C at 7°C/min, with a 5 min hold at the maximum temperature. We used Helium as carrier gas at a flow rate of 1.5 ml/min and set injector and detector temperatures at 200°C and 250°C, respectively. The GC was operated in splitless mode. Sulphur volatiles were identified by matching the Linear Retention Index, LRI, values with authentic standards; alkanes (C5–C25) were used to calculate the values (Rouseff et al. 2008).

The airborne concentration of DMDS within the arms of the 4-choice olfactometer was determined using a fixed volume headspace procedure. After heating a 2.0-ml gas tight glass syringe in a 50°C oven, 1 ml of air from the olfactometer arm containing synthetic DMDS was drawn up and then directly injected into the gas chromatograph described above using leather gloves. A calibration curve covering the range 0.03–0.30 ng was constructed, using 0.3 μl injections of DMDS solutions that were 100–1000 ng/ml. Specifically, 0.3 μl samples of DMDS solutions (100–1000 ng/ml) in methanol were injected in triplicate and peak areas were compared. Linearity was established with a correlation coefficient of $R^2 = 0.95$.

Effect of DMDS on *D. citri* in a small plot field trial

A small plot trial was conducted to test the effect of synthetic DMDS released from polyethylene vials on population densities of *D. citri* in the field. The treatments compared were plots treated with DMDS vs. untreated control plots. Fifteen ml of synthetic DMDS (described above) was formulated per polyethylene vial (Alpha Scents, West Linn, OR). Vials were attached to tree branches with steel wire 2.0 m above ground level. Two vials were attached per tree on opposite sides. Control plots were left completely untreated and no additional insecticides were sprayed for *D. citri* during the course of the experiment. The experiment was initiated on 25 April 2009. Treatments were arranged as a randomized complete block design with five 0.16 ha replicates per treatment in an 8-year-old orange orchard [(*Citus sinensis* [L.]) var 'Valencia.'] in Clermont, FL, USA. Trees were planted on a 3.0×6.0 m spacing and average canopy height was 4.0 m. Replicate plots were separated by 60 m and blocks of treatments were separated by 80 m. Populations of *D. citri* in treatment and control plots were sampled before treatments were applied and then weekly for 28 days thereafter. On each sampling date, 10 trees were sampled for *D. citri* per plot. Each tree was sampled by vigorously tapping three branches directly over a horizontally placed 210×297 mm plastic white sheet. All adult *D. citri* found on the sheet following branch agitation were counted and recorded.

Data analyses

For the 4-choice olfactometer data, the number of *D. citri* remaining at the release point or contacting the source of volatiles was compared between treatments by one-way analysis of variance (ANOVA) followed by Tukey's HSD test ($P < 0.05$, SAS Institute Inc. 2003). Y-tube olfactometer data were analysed by chi-square tests to compare between possible binary psyllid responses ($P < 0.05$). For the field experiment, the mean number of psyllids counted in control vs. DMDS-treated plots on each sampling date was compared by Student's *t*-tests.

Results

Response of *D. citri* to citrus with guava volatiles or DMDS in 4-choice olfactometer

When presented with clean air, *D. citri* responded equally to each of the four arms of the olfactometer ($F = 0.8$; d.f. = 3, 76; $P = 0.5$), indicating no positional bias in the bioassays. Specifically, 20%, 19%, 22%, and 16% of the tested psyllids (n = 200) oriented to each of the four extending arms of the olfactometer, while nearly 24% remained in the central orifice insertion point.

Significantly ($F = 15.2$; d.f. = 7, 112; $P < 0.0001$) more *D. citri* did not move from the release point in treatments in which intact guava, crushed guava, or DMDS were co-presented with citrus compared with when intact citrus was presented alone (fig. 1). The

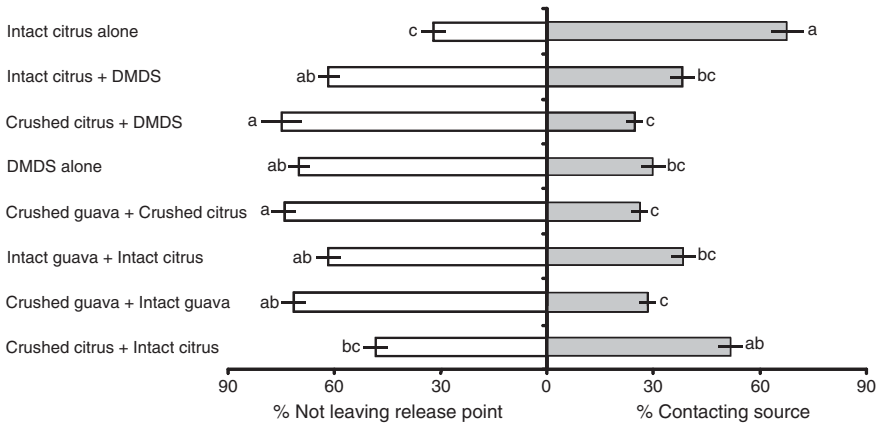

Fig. 1 Responses of *D. citri* to volatiles emanating from intact citrus; crushed citrus and DMDS; DMDS only; crushed guava and crushed citrus; intact guava and intact citrus; crushed guava and intact guava; and crushed citrus and intact citrus in the 4-choice olfactometer. Grey bars represent the percentage of *D. citri* attracted to the source of volatiles and white bars represent the percentage not moving from the release point. White or grey bars followed by the same letters are not significantly different (Tukey's HSD test, $P < 0.05$).

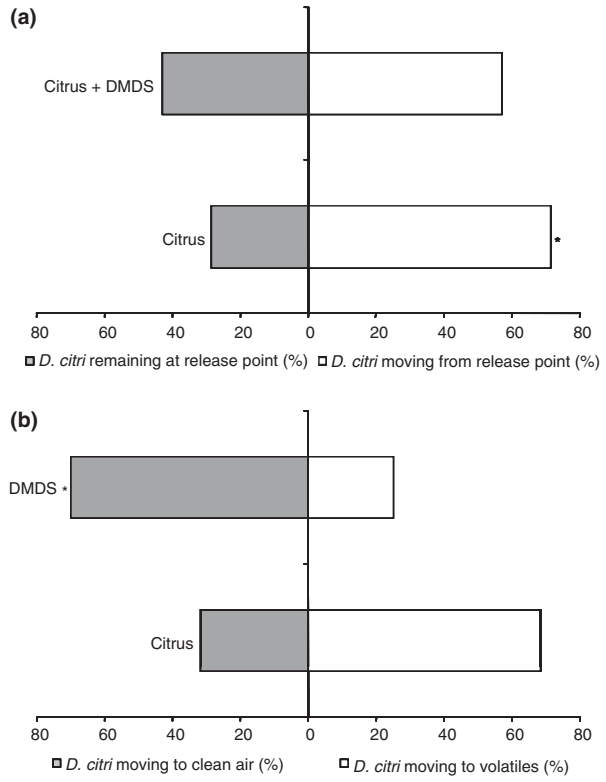

Fig. 2 Responses of *D. citri* in a Y-tube olfactometer when presented with volatiles emanating from intact citrus alone (Citrus) or intact citrus and DMDS (Citrus + DMDS) released from both arms of the olfactometer. Grey bars represent the percentage of *D. citri* remaining at the release point and white bars represent the percentage of *D. citri* contacting the source of volatiles (a); Responses of *D. citri* when presented with laboratory air in one arm of the olfactometer vs. volatiles emanating from intact citrus (Citrus) or DMDS in mineral oil (DMDS) in the other arm (b). Grey bars represent the percentage of *D. citri* not moving from the release point and white bars represent the percentage attracted to the source of volatiles. Significant differences obtained by chi-square analysis are indicated by an asterisk.

airborne concentration of DMDS quantified in the 4-choice olfactometer was 107 pg/ml. The percentage of *D. citri* not moving from the central orifice ranged between 62% and 75% when guava or DMDS were co-presented with citrus compared 32–48% when citrus was presented alone (fig. 1). When a combination of crushed and intact guava leaves were a source of volatiles, approximately 71% of the 210 *D. citri* tested did not move from the release point. When volatiles from intact or crushed guava were co-released with intact or crushed citrus, respectively, significantly fewer *D. citri* were found in the extending arms of the olfactometer than when intact citrus flush was presented alone (fig. 1). Significantly more *D. citri* were found at the insertion point of the olfactometer when exposed to synthetic DMDS alone than when exposed to volatiles from citrus flush alone (fig. 1). When volatiles from intact guava and intact citrus were presented simultaneously, we observed that the *D. citri* captured in the glass traps appeared knocked down and motionless.

Response of *D. citri* to citrus and DMDS in Y-tube olfactometer

The proportion of *D. citri* responding to citrus volatiles co-released with DMDS was significantly ($\chi^2 = 6.6$, d.f. = 1, P = 0.01) lower than the proportion responding to citrus volatiles alone (fig. 2a). Significantly ($\chi^2 = 5.0$, d.f. = 1, P = 0.02) more *D. citri* chose the arm of the Y-tube with throughput of clean air compared with the arm with DMDS (fig. 2b). However, significantly ($\chi^2 = 7.6$, d.f. = 1, P = 0.01) more *D. citri* chose the arm with citrus volatiles compared with clean air (fig. 2b).

Response of *D. citri* to citrus and DMDS-related volatiles in Y-tube olfactometer

Significantly ($\chi^2 = 6.1$, d.f. = 1, P = 0.02) more *D. citri* chose the arm of the Y-tube containing volatiles from intact citrus flush compared with the clean air control (fig. 3). The response of *D. citri* to the arm with volatiles from citrus leaves alone vs. the arm with clean air was nearly identical to that when citrus leaves were co-presented with either dipropyl disulphide, ethyl-1-propyl disulphide, or diethyl disulphide (fig. 3). However, when citrus leaves were concurrently presented with DMDS, significantly ($\chi^2 = 8.0$, d.f. = 1, P = 0.01) more *D. citri* chose the arm with clean air compared with the arm receiving the volatile treatment (fig. 3).

Sulphur-based compounds from intact and crushed guava and citrus

Pulsed flame photometric detector analyses of static head-space volatiles revealed the presence of carbon disulphide (CS_2) in intact samples of both guava and citrus. Dimethyl sulphide (DMS) was also released by intact citrus flush. Gentle crushing of leaf samples resulted in a significant immediate reduction in CS_2 production in both guava and citrus but triggered an escalated increase in production of DMS in guava (fig. 4). The amount of DMS produced by the crushed citrus flush was relatively small at first but increased over time (fig. 4). In addition, methanethiol (CH_3SH) and DMDS were produced by crushed guava leaves in appreciable quantities (fig. 4).

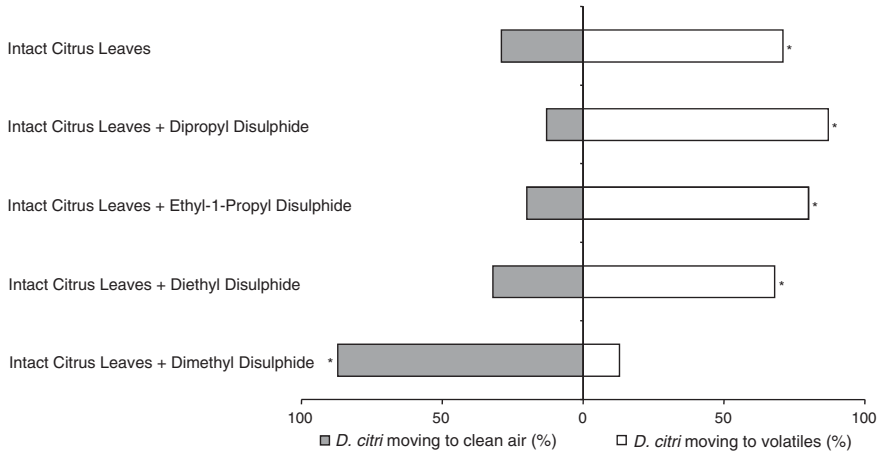

Fig. 3 Responses of *D. citri* to volatiles in a Y-tube olfactometer when presented with laboratory air in one arm of the olfactometer vs volatiles emanating from intact citrus combined with or without various disulphide compounds released from mineral oil (DMDS) in the other arm. Grey bars represent the percentage of *D. citri* entering the arm with clean air and white bars represent the percentage of *D. citri* entering the arm with the source of volatiles. Significant differences obtained by chi-square analysis are indicated by an asterisk.

Effect of DMDS on *D. citri* in small plot field trial

The numbers of adult *D. citri* counted in treated and control plots were nearly identical before treatments were applied (fig. 5). Significantly (t = 2.3–3.1, d.f. = 1, $P < 0.05$) fewer psyllids were found in treatment than control plots for up to 3 weeks following treatment application (fig. 5). By 28 days after treatment application, psyllid densities were nearly identical in treatment and control plots and dispensers appeared completely depleted of the DMDS active ingredient (fig. 5).

Discussion

Volatile chemicals released by plants can repel herbivores (Pickett et al. 1992; Agrawal and Karban 1999) and deter their feeding (Jackson et al. 1996; Dugravot et al. 2003). Our results provide evidence that guava leaf volatiles inhibit the response of *D. citri* to its normally attractive host plant volatiles. As expected, *D. citri* were attracted to volatiles released by citrus leaves in our laboratory olfactometer assays (Wenninger et al. 2009). However, when guava leaf volatiles were simultaneously presented with citrus leaf volatiles, the response of *D. citri* was significantly reduced. Furthermore, DMDS, which is a potent headspace volatile released by guava leaves upon wounding (Rouseff et al. 2008), also inhibited attraction to host plant volatiles. However, a small series of disulphide compounds homologous to DMDS did not affect the response of *D. citri* to normally attractive citrus volatiles when co-presented at the same solution dosage at which DMDS was active (Fig. 3). These data suggest that the activity of DMDS on the behaviour of *D. citri* is unique and not shared by all disulphide compounds. We quantified the airborne concentration of DMDS that induced the behavioural effect in the 4-choice olfactometer and found it to be 107 pg/ml. It has been suggested that toxic metabolites produced and released by guava may explain its repellent effect against *D. citri* when this plant is intercropped with citrus (Zaka et al. 2010), but the mode of action was unknown. We suggest that DMDS, which is released by guava but not citrus leaves, may partially explain this effect on psyllid behaviour.

In assays in which *D. citri* entered the olfactometer stage in response to a combination of volatiles from intact guava and citrus, the captured psyllids were found knocked down and appeared dead. It is possible that this effect may be attributed to the neurotoxicity of DMDS (Auger et al. 1999; Dugravot et al. 2002) or other guava or citrus volatiles. Jackson *et al.* (1996) also reported that aphids exposed to high concentrations of monoterpenes were intoxicated and suggested it was consistent with the hypothesis that monoterpenes inhibit acetylcholinesterase, which is vital for impulse transmission in insects (van Oosten et al. 1990). Carbon disulphide (CS_2), another known insect neurotoxin and fumigant (Tabacova and Balabaeva 1980; Clerici and Fetcher 1991), was also detected in the static head-space volatiles of both guava and citrus. CS_2 was the only

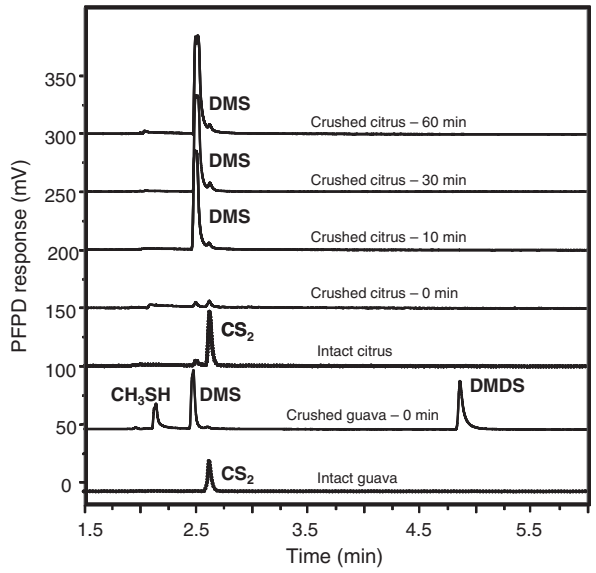

Fig. 4 Comparison of sulphur volatiles from guava and citrus flush. Chromatograms depict sulphur volatiles from intact and crushed guava compared with those of intact and crushed citrus. Static headspace volatiles were collected from either intact guava or citrus flush after equilibrating the samples at ambient laboratory conditions for ~30 min, or at various durations after mechanical damage (0, 10, 30 or 60 min). GC-PFPD responses (mV) are shown on the *y*-axis and retention time (min) on the *x*-axis.

PFPD-detected major compound from intact guava while CS_2 and dimethyl sulphide (DMS) were detected from citrus. It is possible that CS_2 may have contributed to the knock-down effect. Psyllids exposed to crushed citrus + intact citrus exhibited a slight, but non-significant reduction in responsiveness compared with those exposed to intact citrus alone (fig. 1), suggesting that damaging citrus leaves may have reduced their attractiveness. This could be attributed to the greater overall volatile release or release of specific inhibitory compounds by citrus in response to wounding.

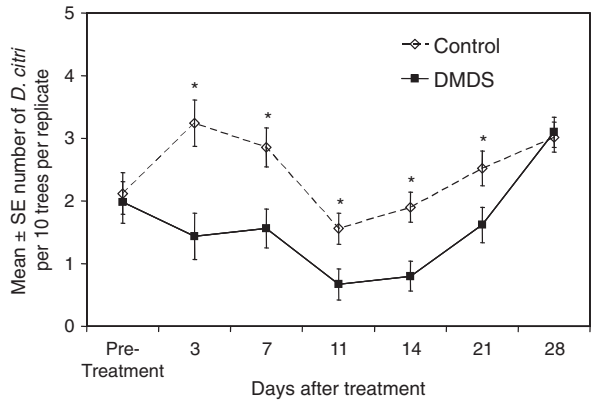

Fig. 5 Average number of *D. citri* obtained per 10 trees per replicate using a beat sheet sampling method in plots treated with synthetic DMDS compared with untreated control plots. Points followed by an asterisk indicate significant differences (*t*-test, $P < 0.05$).

Our small plot field experiment confirmed the results of our laboratory olfactometer assays. Deployment of synthetic DMDS from polyethylene vials reduced populations of *D. citri* in an unsprayed citrus orchard for up to 3 weeks following deployment. Given that population densities were equivalent amongst plots prior to the deployment of DMDS treatments, we hypothesize that DMDS repelled adult *D. citri* from treated plots. However, we cannot exclude the possibility that a proportion of the *D. citri* populations in DMDS-treated plots may have been reduced due to direct intoxication. By the fourth week, there was no remaining DMDS in the polyethylene vials, which likely explains why populations were once again equivalent in treated and control plots. Given the volatility of DMDS, one of the main obstacles to the development of a practical DMDS formulation for *D. citri* management will be development of a slow-release device that maintains the chemical above a behaviourally active threshold for long periods. The polyethylene vials evaluated in this initial proof-of-concept investigation will likely not be economically practical for releasing DMDS for control of *D. citri*. Both the number of dispensers required per acre (~200) as well as the amount of active ingredient required per three weeks (~3 kg) would likely be economically prohibitive for a hand applied dispenser. Furthermore, the dispensers evaluated in this study resulted in a ~2/3 decrease in field populations of *D. citri*, which would be insufficient for effective control of this pest as a stand alone treatment. Another logistical hurdle to developing DMDS into a practical psyllid management tool is the chemical's strong and unpleasant odour. This may render field application difficult and potentially limit the use of DMDS depending on fruit harvesting schedules or proximity to urban areas. Ideally, a slow-release dispenser should be developed that could achieve 150–200 days of behaviourally efficacious release, as is common with current mating disruption formulations of pheromones (Stelinski et al. 2005). Although it is unlikely that a DMDS-based repellent would replace insecticides for control of *D. citri*, it is possible that insecticide use could be reduced by supplemental treatments of a repellent. *D. citri* populations are much more prevalent on crop borders (Boina et al. 2009) and thus targeted applications of DMDS to

those areas may be immediately useful with a sub-optimal dispenser, such as the polyethylene vials evaluated herein.

In summary, volatiles from guava inhibit the response of *D. citri* to citrus host plant volatiles. Our results also suggest that DMDS has behavioural activity against *D. citri*. The current intense use of broad spectrum insecticides against *D. citri* is economically non-sustainable and will lead to development of resistance and environmental contamination. Organophosphates, carbamates and pyrethroids are now routinely applied for *D. citri* management six to eight times annually in Florida (Srinivasan et al. 2008). Alternatives to broad spectrum insecticides for *D. citri* management are needed for commercial citrus production to remain viable in the USA, Brazil and other citrus-producing regions of the world. Development of a repellent for *D. citri* control may offer a potential alternative. DMDS, a guava-released metabolite, appears to be a potential candidate repellent for *D. citri*. Our current on-going efforts include formulating DMDS into controlled release devices for extended release of the chemical in the field. Control of *D. citri* with behavioural modification may be one potential tool for management of this plant disease vector.

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