

## ORIGINAL CONTRIBUTION

**Repellency and toxicity of plant-based essential oils and their constituents against *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae)**

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**Abstract**

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, vectors *Candidatus* Liberibacter asiaticus (Las), the presumed causal agent of huanglongbing. *D. citri* generally rely on olfaction and vision for detection of host cues. Certain plant volatiles and plant-derived essential oil products are known to repel several arthropod species and are considered minimum-risk pesticides. We examined the effect of five essential oils and eight chemicals previously reported to have activity against various insect species on *D. citri* behaviour in a two-port divided T-olfactometer in an effort to identify repellents for further consideration and testing as crop protectants for *D. citri*. Volatiles from essential oils of coriander, lavender, rose, thyme, tea tree oil and 2-undecanone, a major constituent of rue oil repelled *D. citri* adults compared with clean air. Also, coriander, lavender, rose and thyme oil inhibited the response of *D. citri* when co-presented with citrus leaves. Volatiles from eugenol, eucalyptol, carvacrol,  $\beta$ -caryophyllene,  $\alpha$ -pinene,  $\alpha$ -gurjunene and linalool did not repel *D. citri* adults compared with clean air. In an effort to isolate the repellents and toxicants from effective essential oils, the headspace components of coriander and lavender oil were analysed by gas chromatography-mass spectrometry and revealed that  $\alpha$ -pinene and linalool were the primary volatiles present in coriander oil while linalool and linalyl acetate were the primary volatiles present in lavender oil. Coriander, lavender and garlic chive oils were also highly toxic to *D. citri* when evaluated as contact action insecticides using a topical application technique. The  $LC_{50}$  values for these three oils ranged between 0.16 and 0.25  $\mu\text{g}/D. citri$  adult while  $LC_{50}$  values for rose and thyme oil ranged between 2.45 and 17.26  $\mu\text{g}/\text{insect}$ .

**Introduction**

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is one of the most economically destructive pests of citrus worldwide. *D. citri* vector the phloem-inhabiting bacterium *Candidatus* Liberibacter asiaticus, which is the presumed causal agent of huanglongbing (HLB) disease in citrus in North America. HLB, otherwise known as citrus

greening disease, is the greatest biotic threat to citrus production worldwide (McClellan and Schwarz 1970; Halbert and Manjunath 2004; Bové 2006). HLB affects plant phloem, causing yellow shoots, mottling, chlorosis, and twig die back that result in rapid tree decline and may ultimately cause tree death. Fruit on diseased trees do not color properly, are sour tasting, and are of reduced size (Capoor 1963; Halbert and Manjunath 2004; Bové 2006; Dagulo

et al. 2010). In areas of the world where HLB is endemic, citrus trees decline and die within a few years and may never produce usable fruit (Halbert and Manjunath 2004).

Primary control of the vector has relied on broad spectrum insecticides (Rogers 2008). However, insecticide use has negatively affected populations of natural enemies and may lead to development of insecticide resistance. Therefore, novel management strategies such as insect repellents, attractants and antifeedents may serve as useful alternatives or supplements to insecticides. Plant-derived essential oils are generally of low-molecular weight and highly volatile (Miresmailli and Isman 2006). These essential oils are often specific to the target species and typically have unique modes of action with low toxicity to non-target organisms. Furthermore, essential oils are often environmentally safe with rapid biodegradation, and are non-toxic to humans and other mammals (Isman 2000). Although repellent and insecticidal properties of plant-derived essential oils have been examined on several hemipterans (aphids, thrips, whiteflies, mealy bug) (Agarwal et al. 2001; Miresmailli and Isman 2006; Cloyd et al. 2009), investigations with *D. citri* are still in a preliminary phase. Therefore, the purpose of this research was to evaluate the repellent and insecticidal activity of selected essential oils or their major constituents on *D. citri* with an aim to identify an effective plant-based repellent and/or insecticide.

## Materials and methods

### Insects

Adult *D. citri* used in behavioural bioassays were obtained from a laboratory culture at the University of Florida Citrus Research and Education Center (Lake Alfred, FL). The culture was established in 2000 from field populations in Polk Co., FL (28.0°N, 81.9°W) prior to the discovery of HLB in FL. The culture is maintained without exposure to insecticides on sour orange (*Citrus aurantium* L.) and 'Hamlin' orange [*C. sinensis* (L.)] seedlings at  $27 \pm 1^\circ\text{C}$ ,  $63 \pm 2\%$  RH, and under a L14 : D10 photoperiod.

### Essential oils and their constituents

Essential oils of coriander ( $\geq 89\%$  purity), garlic (unknown purity), rose (unknown purity), thyme ( $\geq 95\%$  purity), and major terpene constituents of clove oil (eugenol, 99% purity), eucalyptus oil (eucalyptol,  $\geq 95\%$  purity), guava oil ( $\alpha$ -gurjunene,

$\geq 97\%$  purity,  $\beta$ -caryophyllene,  $\geq 80\%$  purity), oregano oil (carvacrol,  $\geq 98\%$  purity), rosemary oil ( $\alpha$ -pinene,  $\geq 99\%$  purity), rue oil (2-undecanone, 99% purity) and coriander oil (linalool,  $\geq 97\%$  purity) were obtained from Sigma-Aldrich Inc., St Louis, MO. The lavender oil (100% purity) and tea tree oil (99% purity) were obtained from The Good Scents Company, Ellicott City, MD. The essential oils were selected based on their previously reported activity against various insect species (Isman 2000; Cloyd et al. 2009; Sukontason et al. 2004; Waliwitiya et al. 2005; Williamson et al. 2007; Sfara et al. 2009; Tarelli et al. 2009).

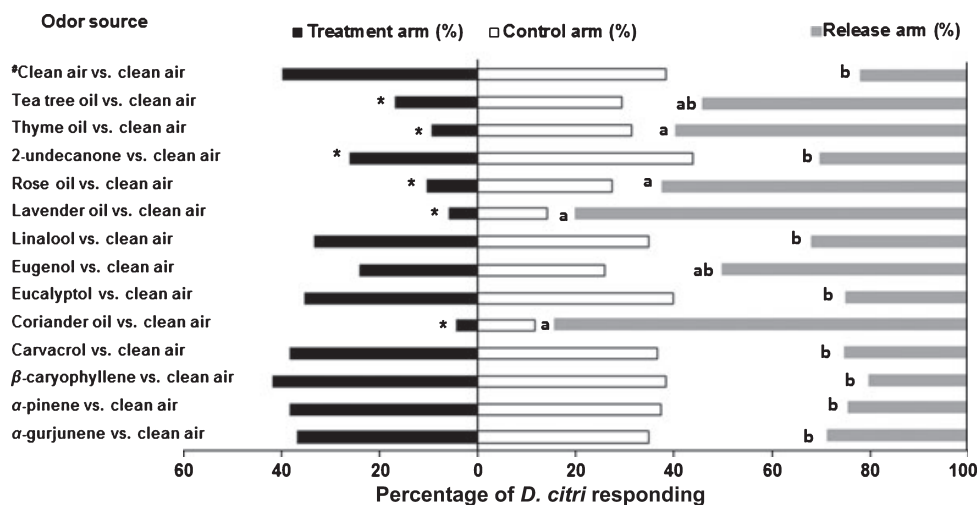
### Behavioural bioassays

A custom-designed two port divided T-olfactometer from Analytical Research Systems (ARS), Inc. Gainesville, FL and thoroughly, described in Mann et al. (2010a) was used to evaluate behavioural response of *D. citri* to essential oil volatiles. The olfactometer consisted of a 30 cm glass tube that is bifurcated into two equal halves with a Teflon strip forming a T-maze. Each half served as an arm of the olfactometer enabling the ACP to make a choice between two potential odour fields. The olfactometer arms were connected to odour sources placed in solid-phase micro-extraction chambers (SPME) (ARS, Gainesville, FL) through Teflon<sup>®</sup>-glass tube connectors. The samples were evaluated at a 5.0 mg/ml dosage. The chemical samples were dissolved in 1 ml ethylene glycol (EG) and pipetted onto a 5 cm Richmond cotton wick (Petty John Packaging, Inc., Concord, NC). The dosage was selected based on our previous investigations with garlic chive essential oil which significantly repelled *D. citri* adults at a minimum dosage of 5.0 mg/ml (Mann et al. 2010a). The 1.0 mg/ml dosage was included to determine if any of the investigated essential oils or chemicals was active at a dosage lower than garlic chive oil. The control treatment consisted of a cotton wick impregnated with 1 ml EG only. The treated and control cotton wicks were wrapped in laboratory tissue (Kimwipes, Kimberly-Clark, Roswell, GA) and placed in a SPME (ARS, Gainesville, FL). A SPME consisted of a straight glass tube (17.5 cm long  $\times$  3.5 cm wide) supported with an inlet and outlet valve for incoming and outgoing air streams, respectively (Mann et al. 2010a). The purified and humidified air was pushed through the SPME via two pumps connected to an air delivery system (ARS, Gainesville, FL). A constant airflow of 0.1 l/min was maintained through both arms of the olfactometer. Purified and

humidified air was pushed through these chambers via two pumps connected to an air delivery system (ARS, Gainesville, FL) (see fig. 1 in Mann et al. 2010a for diagram). The olfactometer was housed within a temperature controlled room and positioned vertically under a fluorescent 900 lux light bulb fixed in a 1.0 × 0.6 × 0.6 m fibre board box for uniform light diffusion. This position took advantage of the negative geotactic and positive phototactic response of *D. citri* (Mann et al. 2010a). The olfactometer inlet adapter was covered with black cloth to facilitate insect movement towards odour sources by using the positive phototactic response of *D. citri* (Mann et al. 2010a).

Prior to tests involving putative repellents, female *D. citri* adults were exposed to clean air vs. clean air or EG vs. clean air in the T-maze olfactometer to verify the absence of positional bias or an effect of EG on psyllid behaviour. An odour source was randomly assigned to one of the arms of the olfactometer at the beginning of each bioassay and was reversed after every 30 insects to eliminate potential of positional bias. A minimum of 120 *D. citri* female adults were examined per treatment combination (four replications of 30 *D. citri*). However, given that more *D. citri* remained at the point of release when exposed to coriander, lavender, rose and thyme oils, than for the other treatments, the number of replications for these treatments was increased to six to obtain a minimum of 30 insects entering the olfactometer per treatment. Female *D. citri* are more

responsive to citrus odours than males (Wenninger et al. 2009); therefore, only female adults were tested in this study. *D. citri* females were released individually into the inlet adapter at the base of the olfactometer. Adults were given 300 s to exhibit a behavioural response by entering either olfactometer arm. The numbers of adults entering the treatment arm, control arm or remaining in the release port or below the T-maze division were recorded. A treatment or control arm choice was recorded when an insect moved into either olfactometer arm by crossing the division in the T-maze. A release arm choice was recorded when an insect remained in the release port or below the T-maze division. All experiments were conducted at 26 ± 1°C and 60 ± 2% RH. The olfactometer and connecting tubes were thoroughly cleaned with 2% soap solution and baked at 200°F between each treatment run. The essential oils and their constituents showing the highest activity against *D. citri* were also evaluated at a lower 1.0 mg/ml dosage. Given that *D. citri* are attracted to citrus odours, the essential oils and the chemicals were also evaluated in the presence of citrus odours at the minimum dosage that repelled *D. citri* when presented opposite to clean air (control arm) in the olfactometer. For this, 2.0 g of fresh citrus leaves (flush) was placed in both the oil sample treatment and control arm of the olfactometer. Citrus vs. citrus was used as the control treatment in this set of experiments. Given that fresh leaves are known to contain higher proportions of secondary compounds



**Fig. 1** Responses of *D. citri* when presented with volatiles emanating from essential oils vs. clean air. Black and white bars are compared against each other within an odour source comparison (within rows) and grey bars are compared among odour sources (among rows). Black bars (*D. citri* moving to treatment arm) followed by \* are significantly different from white bars (*D. citri* moving to control arm) ( $\chi^2$  test,  $P < 0.05$ ). †Control treatment. Grey bars (*D. citri* not moving from point of release) followed by same letters are not significantly different (Tukey's HSD test,  $P < 0.05$ ).

(Hrutfiord et al. 1974) and *D. citri* are primarily associated with new growth (Catling 1970), we used fresh leaf flush [immature leaves of the growing shoots (Hall and Albrigo 2007)] for *D. citri* behavioural assays. The bioassay procedures were otherwise identical to those described for the essential oil evaluations vs. clean air.

### Chemical analyses

To explain the repellent and toxic activities of essential oils, the head-space volatiles from the most effective essential oils (lavender and coriander) were identified using a static solid-phase micro-extraction (SPME) technique described in Rouseff et al. (2008). Briefly, a 200 mg oil sample was weighed into a 40 ml septum-sealed glass vial. The sample was allowed to equilibrate at ambient laboratory conditions for 30 min. Accumulated volatiles were collected from the glass vials for 1 min post exposure to ambient laboratory conditions. The volatiles were collected using a 75  $\mu\text{m}$  carboxen-polydimethylsiloxane Stable Flex<sup>®</sup> SPME fiber (Supelco, Bellefonte, PA). At least two replicates of each volatile sample were analysed. The collected volatiles were analysed and identified with a Perkin/Elmer<sup>®</sup> Clarus 500 quadrupole mass spectrometer (GC-MS). The GC-MS was equipped with Turbo Mass software (Perkin/Elmer, Shelton, CT) and a 60 m  $\times$  0.25 mm, i.d.  $\times$  0.50  $\mu\text{m}$  film thickness, Restek (Stabilwax) capillary column.

Helium was used as the carrier gas at a constant flow of 2 ml/min. The source was kept at 180°C, and the transfer line and injector were maintained at 240°C. The oven was programmed from 40 to 240°C at 7°C/min. We matched mass spectra with NIST 2005 version 2.0 standard spectra (NIST, Gaithersburg, MD) and identifications with spectral fit values equal to or greater than 800 were considered positive. Confirmation of standardized retention values (LRI or Kovats) were also acquired for positive identifications. Authentic standards were used to confirm identifications when available.

### Contact toxicity bioassays

The essential oils of lavender, coriander, rose and thyme that showed the greatest repellent activity against *D. citri* were also evaluated for their insecticidal activity. Given that garlic chive essential oil was found to significantly repel *D. citri* adults in our previous investigations (Mann et al. 2010a), it was also included in the current toxicity evaluations. Seven serial dilutions ranging between 0% and 2%

of each test essential oil or chemical was prepared by dissolving the test chemical in acetone. All test essential oils and chemicals were readily soluble in acetone showing no precipitation or phase partitioning upon dilution. To evaluate insecticide contact activity, 0.4  $\mu\text{l}$  of each serial dilution of essential oil or an acetone only (control) treatment were applied from lower to higher concentration using a Hamilton micro-applicator to the dorsal mesothorax of adult psyllids. Psyllids were anesthetized with CO<sub>2</sub> for about 2 min to facilitate insecticide application and transfer into Petri dishes. Prior to bioassays with essential oils, *D. citri* adults were exposed to untreated Petri dishes (with 1.5% solidified agar beds, filter paper and leaf discs only) to verify that CO<sub>2</sub> anesthetization did not impact psyllid mortality. Treated psyllids were transferred to 60-mm-diameter plastic disposable Petri dishes (Thermo Fisher Scientific, Waltham, MA) containing a citrus leaf disc placed on 1.5% solidified agar beds to prevent desiccation as described by Boina et al. (2009). Each solidified agar bed was covered with a Whatman no. 1 filter paper to prevent insects from moving under the leaf discs and sticking to the agar. We observed no effect of CO<sub>2</sub> anesthetization on psyllid mortality as insect survival in control treatments was always > 90%. Mortality of *D. citri* was scored 48 h after exposure to treatments. Insects that were unable to move when prodded were considered dead and included in mortality counts. For each concentration, a minimum of 80 insects were examined. All insects were held at 25  $\pm$  2°C during the bioassays.

### Data analysis

For assays in which putative repellent treatments were presented in the T-maze olfactometer with or without citrus and versus clean air, the number of *D. citri* remaining at the release point and not entering the olfactometer was compared between treatments by one way analysis of variance (ANOVA) followed by Tukey's HSD test ( $P < 0.05$ ). ANOVA was performed on four replicates. For *D. citri* leaving the release arm, the number of *D. citri* choosing the control arm vs. the treatment arm was compared with chi-square analysis at  $P < 0.05$ . The data from all four replicates were combined for the chi-square analysis. Analysis of mortality data was conducted using a probit analysis. Descriptive statistics and LC<sub>50</sub> values were estimated using SAS (Cary, NC) statistical software. Abbott's transformation was performed as part of the PROBIT procedure to correct for control mortality for instances when it occurred at

acceptable levels (< 10%). The LC<sub>50</sub> values were considered significantly different (P < 0.05) if the 95% confidence intervals did not overlap. Analysis was conducted on four replications with 20 insects per replication.

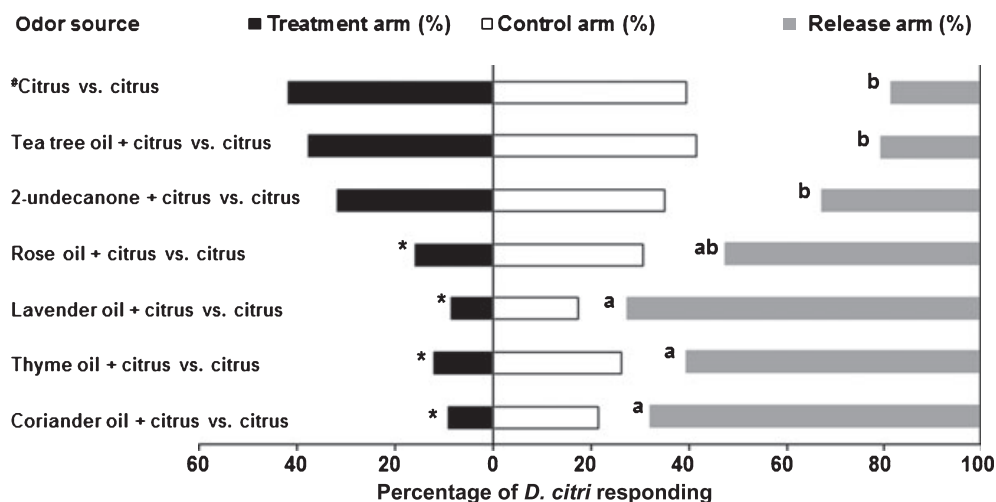
**Results**

**Behavioural bioassays**

Up to 78% of tested *D. citri* entered the olfactometer in response to light and negative geotaxis when clean air was presented in both arms (fig. 1). Furthermore, there were no significant differences between the numbers of *D. citri* that entered each arm when clean air was presented vs. clean air ( $\chi^2 = 0.04$ ; d.f. = 1; P = 0.83), or EG was presented vs. clean air ( $\chi^2 = 0.27$ ; d.f. = 1; P = 0.6). Significantly (F = 12.50; d.f. = 13.63; P < 0.0001) more *D. citri* did not move from the release point in treatments in which odours of coriander, lavender, rose or thyme oil were co-presented with clean air compared with when clean air was presented alone (control treatment) (fig. 1). There were no significant differences between the responses to these four essential oils with respect to the number of *D. citri* remaining at the release point. The percentage of *D. citri* not moving from the release point when challenged with any of these four essential oils ranged between 65% and 83% as compared to 22% in clean air.

More *D. citri* entered the arm receiving clean air than the arm receiving the odours of coriander ( $\chi^2 = 6.53$ ; d.f. = 1; P = 0.01), lavender ( $\chi^2 = 7.71$ ; d.f. = 1; P = 0.005), rose ( $\chi^2 = 7.33$ ; d.f. = 1; P = 0.006) or thyme oil ( $\chi^2 = 17.85$ ; d.f. = 1; P < 0.0001) when volatiles from these essential oils were released vs. clean air (fig. 1). Furthermore, more *D. citri* entered the arm receiving clean air than the arm receiving the odours of tea tree oil ( $\chi^2 = 7.68$ ; d.f. = 1; P = 0.01) or 2-undecanone ( $\chi^2 = 5.22$ ; d.f. = 1; P = 0.02) (fig. 1). There were no significant differences between the number of *D. citri* entering the olfactometer arm containing eugenol, eucalyptol, carvacrol,  $\beta$ -caryophyllene,  $\alpha$ -pinene,  $\alpha$ -gurjunene or linalool vs. clean air (fig. 1). Roughly equivalent numbers of *D. citri* left the release arm and there was no significant difference between number of *D. citri* entering the treatment arm or control arm when coriander, lavender, rose or thyme oil were evaluated at a lower 1.0 mg/ml dosage tested.

More (F = 12.50; d.f. = 6,21; P < 0.0001) *D. citri* remained at the release point when the odours of coriander + citrus, lavender + citrus, or thyme + citrus were presented vs. citrus compared with when citrus was presented vs. citrus (fig. 2). The percentage of *D. citri* not moving from the release point for these three essential oils in the presence of citrus ranged between 53% and 75%. Furthermore, significantly more *D. citri* entered the arm receiving odours from citrus than the arm receiving odours



**Fig. 2** Responses of *D. citri* when presented with volatiles emanating from essential oils in the presence of citrus odours. Black and white bars are compared against each other within an odour source comparison (within rows) and grey bars are compared among odour sources (among rows). Black bars (*D. citri* moving to treatment arm) followed by \* are significantly different from white bars (*D. citri* moving to control arm) ( $\chi^2$  test, P < 0.05). †Control treatment. Grey bars (*D. citri* not moving from point of release) followed by same letters are not significantly different (Tukey's HSD test, P < 0.05).



from coriander + citrus ( $\chi^2 = 6.08$ ; d.f. = 1;  $P = 0.01$ ), lavender + citrus ( $\chi^2 = 3.90$ ; d.f. = 1;  $P = 0.04$ ), thyme+citrus ( $\chi^2 = 5.56$ ; d.f. = 1;  $P = 0.02$ ) or rose + citrus ( $\chi^2 = 5.78$ ; d.f. = 1;  $P = 0.02$ ) (fig. 2). However, the numbers of *D. citri* entering the arm receiving 2-undecanone + citrus or tea tree oil + citrus were not significantly reduced compared with response to citrus alone.

### Chemical analyses

The primary chemicals identified from coriander oil were  $\alpha$ -pinene, linalool, p-cymene, limonene,  $\gamma$ -terpinene, and  $\beta$ -pinene (table 1). More than 80% of coriander oil consisted of various terpenes and lacked

any identifiable esters whereas lavender oil contained three major esters which comprised approximately 16% of the total volatiles and only 42% of total volatiles consisted of various terpenes. The major chemicals identified from lavender oil were linalool, *cis*-ocimene, *trans*-ocimene, eucalyptol, linalyl acetate,  $\beta$ -myrcene, octan-3-one, limonene,  $\alpha$ -pinene and 4-terpineol (table 1). Linalool,  $\alpha$ -pinene, camphene,  $\beta$ -pinene,  $\beta$ -myrcene, limonene, p-cymene were found in both coriander and lavender oils (table 1).

### Contact toxicity bioassays

The three essential oils that showed the highest repellent activity against *D. citri* adults were also

**Table 1** GC-MS identification of major volatiles from coriander and lavender essential oils

Essential oil	Chemical	CAS no.	Obs wax column LRI	Lit wax column LRI	Retention time	% of total
Coriander	$\alpha$ -pinene*	80-56-8	1037	1038	8.07	37.45
	Camphene	79-92-5	1079	1085	8.98	8.38
	$\beta$ -pinene*	127-91-3	1119	1131	9.87	2.94
	Sabinene	3387-41-5	1133	1127	10.19	1.36
	$\beta$ -myrcene*	123-35-3	1173	1171	11.07	1.97
	Limonene*	138-86-3	1216	1220	12.01	10.30
	$\gamma$ -terpinene*	99-85-4	1262	1267	13.01	7.58
	p-cymene*	99-87-6	1292	1297	13.63	11.77
	$\alpha$ -terpinolene*	586-62-9	1300	1306	13.81	0.59
	Z-linalool oxide*	5989-33-3	1467	1467	17.12	0.60
	Linalool*	78-70-6	1561	1558	18.86	15.09
	Camphor	76-22-2	1563	1532	18.90	1.99
	Lavender	Hexyl methyl ether	4747-07-3	958	958	6.50
$\alpha$ -pinene*		80-56-8	1031	1031	7.95	2.55
$\alpha$ -thujene		2867-05-2	1035	1035	8.04	1.04
Camphene		79-92-5	1077	1077	8.96	1.84
$\beta$ -pinene*		127-91-3	1119	1119	9.87	0.51
$\delta$ -3-carene*		13466-78-9	1162	1162	10.84	1.59
$\beta$ -myrcene*		123-35-3	1173	1173	11.09	4.31
$\alpha$ -phallandrene		99-83-2	1180	1180	11.22	0.71
Limonene*		138-86-3	1215	1215	12.00	3.37
Eucalyptol*		470-82-6	1228	1228	12.29	11.70
<i>Cis</i> -ocimene*		3779-61-1	1246	1246	12.67	14.61
<i>Trans</i> -ocimene*		3338-55-4	1264	1264	13.06	10.08
Octan-3-one		106-68-3	1274	1274	13.26	6.02
Hexyl acetate		142-92-7	1287	1287	13.53	1.44
p-cymene*		99-87-6	1290	1290	13.60	1.47
Octen-3-yl acetate		2442-10-6	1390	1390	15.63	1.69
1-Octen-3-ol		3391-86-4	1461	1461	17.00	0.68
Linalool*	78-70-6	1561	1561	18.86	19.91	
Linalyl acetate	115-95-7	1575	1575	19.12	12.73	
4-terpineol*	562-74-3	1634	1634	20.17	2.55	

Identification was based on comparisons of retention times with standard and spectral data from Nist05 Libraries.

\*Authentic standards were used to confirm identifications of the chemicals.

Obs wax column LRI; Linear retention index values observed for wax column; Lit wax column LRI; Linear retention index values reported in literature for wax column.

**Table 2** Mortality of *D. citri* after topical treatment with various essential oils dissolved in acetone

Essential oil	LC <sub>50</sub> ( $\mu\text{g}/\text{insect}$ ) (95% confidence limits)	Slope $\pm$ SE
Garlic chive	0.17 (0.08–0.43)ab	1.75 $\pm$ 0.19
Lavender	0.16 (0.13–0.18)a	2.44 $\pm$ 0.25
Coriander	0.25 (0.19–0.32)b	1.63 $\pm$ 0.35
Rose	2.45 (1.29–11.76)c	1.55 $\pm$ 0.36
Thyme	17.26 (3.12–54.81)c	0.68 $\pm$ 0.19

<sup>a</sup>The LC<sub>50</sub> values were considered significantly different ( $P < 0.05$ ) if the 95% confidence intervals did not overlap.

<sup>b</sup>The LC<sub>50</sub> values with same letters are not significantly different.

toxic through contact activity. Lavender and thyme oils exhibited the lowest (0.16  $\mu\text{g}/\text{insect}$ ) and the highest (17.26  $\mu\text{g}/\text{insect}$ ) LC<sub>50</sub> values, respectively (table 2). There were no significant differences between LC<sub>50</sub> values of lavender and garlic oils. However, coriander oil generated a significantly higher LC<sub>50</sub> value than lavender oil which was comparable to the LC<sub>50</sub> value of garlic oil (table 2). Both rose and thyme oils exhibited significantly higher LC<sub>50</sub> values than garlic, lavender, and coriander oils (table 2).

## Discussion

The current results indicate that essential oils of coriander, lavender, rose and thyme suppressed the response of *D. citri* to normally attractive citrus odours. Furthermore, these oils were toxic to *D. citri* in contact bioassays. Each essential oil evaluated herein has been previously reported to repel or kill various insect species including hemipterans (Obeng-Ofori and Reichmuth 1997; Sukontason et al. 2004; Williamson et al. 2007; Zhang et al. 2004; Cloyd et al. 2009; Dolan et al. 2009; Mann et al. 2010a,b). However, only four of the 13 evaluated essential oils inhibited the response of *D. citri* to its normally attractive citrus host plant volatiles. Furthermore, garlic chive, coriander and lavender essential oils were more toxic to *D. citri* than rose or thyme essential oils. These results indicate specific activity (repellency or toxicity) of garlic, lavender and coriander oils against *D. citri*.

The repellent properties of the tested essential oils is not unexpected given that essential oil products are generally considered broad spectrum because of multiple active ingredients and modes of action (Chiasson et al. 2004). Essential oils may affect the cuticle of soft-bodied insects such as aphids, white-

flies, thrips and psyllids more than that of hard-bodied insects due to lesser sclerotization (Isman 1999; Chiasson et al. 2004). Furthermore, essential oils are known to reduce growth and fecundity of insects and act as antifeedants and moulting inhibitors (Arnason et al. 1989). The mode of action of essential oils is presumed to be interference with the neuro-modulator octopamine (Enan 2001; Kostyukovsky et al. 2002; Waliwitiya et al. 2005) or GABA-gated chloride channels through fumigant activity (Quarles 1996). However, the exact mode of action is still unclear (Isman 1999).

Carvacrol, eugenol, 2-undecanone,  $\alpha$ -gurjunene,  $\beta$ -caryophyllene, and  $\alpha$ -pinene are the major constituents of oregano, clove, rue, guava, and rosemary oils, respectively. None of these chemicals affected the behaviour of *D. citri* at the highest dosage tested despite the reported activity of these essential oils against several insect species (Lee et al. 1997; Isman 2000; Tripathi et al. 2003; Waliwitiya et al. 2005; Miresmailli et al. 2006; Gruber et al. 2009). Similarly, linalool or  $\alpha$ -pinene did not affect the behaviour of *D. citri* at the highest dosage tested even though these were the major constituents of lavender and coriander oils, both of which were highly active against *D. citri*. Therefore, it appears that linalool and  $\alpha$ -pinene do not explain the repellent and toxic activities of these two essential oils. Furthermore, linalool is a minor component (< 2.3%) of citrus leaf, peel and fruit juice head space (Ahmad et al. 2006; Onagbola et al. 2010). Linalool and  $\alpha$ -pinene have been reported to repel or kill several herbivore insect species including hemipterans (Ngho et al. 1998; Hori 1999; Ukeh et al. 2007; Sfara et al. 2009). Conversely, Patt and Setamou (2010) identified linalool as a possible attractant of *D. citri*; however, no evidence of attraction to linalool was observed at the dosage tested herein. Similarly, other constituents of coriander and lavender oils such as,  $\alpha$ -terpeniol, terpinolene, p-cymene, and eucalyptol, previously reported to repel or kill arthropods (Rice and Coats 1994; López et al. 2008; Bleeker et al. 2009; Kaufman et al. 2010; Mann et al. 2010a; b), may or may not be active against *D. citri*. All of the major chemicals identified from coriander oil and lavender oil have been identified previously by Gil et al. (2002) and Shellie et al. (2002). The toxicity of garlic chive oil may have been due to production of sulphur volatiles such as dimethyl trisulfide, dimethyl disulfide and allyl methyl disulfide, which are known to inhibit the response of *D. citri* to attractive host plant volatiles (Mann et al. 2010a). The mechanism of toxicity of coriander and lavender

oil to *D. citri* is unknown, but may be due to the terpene alcohol, linalool, or other terpene chemicals as reported for several other insects (Zou and Cates 1997; Isman 2000; López et al. 2008; Cloyd et al. 2009).

Given that the active ingredients of essential oils are highly volatile, formulating these oils into controlled release devices is necessary for practical pest control applications (Scher 1984; Tarelli et al. 2009). Our current efforts are focusing on quantifying the airborne concentrations of these essential oils found to have behavioural activity against *D. citri* that are required to induce the effect. Our current results suggest that garlic chive, lavender, and coriander essential oils should be further investigated as possible repellents or insecticides against *D. citri*. Current management of *D. citri* in commercial citriculture requires six to eight applications of broad spectrum neurotoxins annually (Rogers and Timmer 2007; Rogers 2008), which destroys natural enemies and may lead to development of resistance. Development of botanical pesticides for *D. citri* could add additional useful tools for the management of this disease vector. Also, such repellents may be useful in organic citrus production, which currently has few available tools for management of *D. citri*.

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