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Attraction and electroantennogram responses of male Mediterranean fruit fly (Diptera: Tephritidae) to six plant essential oils

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Abstract
The present study investigated the differential attractiveness of six natural essential oils in comparison with trimedlure to (1) sterile males Mediterranean fruit fly, *Ceratitis capitata* (Wied.), in laboratory and field cage bioassays conducted in Florida and (2) wild males in field tests conducted in Honduras. Male electroantennogram (EAG) response to the volatiles emitted from the essential oils, was recorded and identify and quantify the terpenoid emissions. Among the oils evaluated, ginger root oil was the most attractive. The amount of α-copaene emitted from oil substrates was not correlated with fly preferences in the bioassays or field tests, nor with the amplitude of EAG response, suggesting that additional chemicals may be necessary to elicit attraction of *C. capitata*. Male preferences among essential oils were positively correlated with the emissions of three male pheromone precursors, β-myrcene, linalool and geraniol, in addition to camphene and α-terpineol.

Keywords: Essential oils, *Ceratitis capitata*, attraction, electroantennography, α-copaene

1. Introduction
Males of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), form mating aggregations (leks) and call (release pheromone) for prospective mates from the foliage of both host and non-host tree species [1, 2]. It has been suggested that plant volatiles, rather than male produced volatiles, play a significant role in male aggregation behavior and site selection for leks [3, 4].
Plant-derived essential oils are obtained typically by steam distillation of fruit, stems, roots or leaves. As sources of volatile chemicals that affect insect behavior [5], essential oils have practical applications in insect pest management. Males of *C. capitata* exposed to ginger root oil, orange oil, or manuka oil have increased mating success compared with males that were not exposed [4, 6, 7]. Exposure to ginger root oil is currently used to increase the mating success of sterile males released for sterile insect technology (SIT) [8]. Volatile compounds from the ginger oil appear to mediate this process, as males do not have to contact the oil to obtain this increased mating success [6, 9].
Natural essential oils contain components that fall into a relatively narrow range of volatilities with low molecular weight, mostly from monoterpenes (C_{10} hydrocarbons) to sesquiterpenes (C_{15} hydrocarbons). Components are either naturally present in plants or are derived from precursors during oil production. Some essential oils were found to be attractive to male *C. capitata* and this led to the development of the currently used male parapheromone [10]. For instance, angelica seed oil (*Angelica archangelica* L.) was found to be attractive to male *C. capitata*, and traps baited with this oil were used for survey and detection for the eradication program in Florida in 1956 [11]. The standard *C. capitata* parapheromone is trimedlure, tert-butyl 4 (or 5)-chloro-2-methylcyclo-hexane-1-carboxylate [12, 13], a synthetic blend which provides a relatively cheap and attractive lure for males. There is no natural source of trimedlure, and males are not observed either to contact or ingest it [14, 15]. Identification of plant-based chemicals that make up an authentic natural model for the parapheromone may lead to development of improved male-targeted attractants and/or feeding stimulants for *C. capitata* [16]. A natural sesquiterpene widely distributed among the plants and derivative essential oils, α-copaene, is suggested to be the main component responsible for attraction of males to these plants/oils [17-19]. However, previous studies of short-range attraction to wood substrates containing α-copaene found no correlation between quantity of this sesquiterpene

and either attraction or electroantennogram (EAG) response [20], indicating that other plant chemicals contribute to male response. Although α-copaene has been the primary chemical targeted as the basis of these biological responses, other sesquiterpenes that co-occur with α-copaene may also contribute alone or as synergists at the behavioral and neurophysiological level, as recently demonstrated for the oriental fruit moth, *Cydia molesta* (Busck) (Lepidoptera: Tortricidae) [21-22].

Reported herein is research that evaluated both long- and short-range attraction of male *C. capitata* to several plant essential oils containing different amounts of α-copaene, using laboratory bioassays and field tests. Electroantennography was also conducted to quantify olfactory response to the volatile emissions from essential oil treatments. We assessed whether the emitted amount of α-copaene influenced the behavioral and physiological responses toward the different essential oils, and discuss the potential role of other terpenoids in attraction of male *C. capitata*.

2. Materials and Methods

2.1 Insects

Sterile male *C. capitata* were obtained from the Programa Moscamed mass rearing facility (El Pino, Guatemala), where they were irradiated as pupae 2 days prior to emergence with 95 Gy of gamma radiation from a Co^{60} source. Irradiated pupae were shipped initially to the USDA-APHIS Medfly Project (Sarasota, FL) and then to the USDA-ARS Subtropical Horticulture Research Station (SHRS) in Miami, FL. Holding conditions at Miami consisted of a 12/12h L/D photoperiod, 25 ± 2 °C, and 75 ± 5% RH. Pupae were placed in collapsible cages (~500 pupae in 30.5 cm^3 cages or ~100 pupae in 20.3 cm^3 cages). After hatching, adult flies were provided with water (2% agar blocks) and food (3:1 mixture of cane sugar and yeast hydrolysate). Flies used for all studies were 5 to 10 d-old (sexually mature) virgin males.

2.2 Test Substrates

Test substrates used in this study were six essential oils, including angelica seed oil (SunRose Cosmetics, New York, NY), cubeb oil, ginger root oil, tea tree oil (Essential Oil India – SAT Group, Kannauj, India), manuka oil (Harmonic Skin Tones, Fallbrook, CA), and Valencia orange oil (Florida Flavors, Lakeland, FL). To provide a positive control and a means to assess relative attraction of the essential oils, we also used liquid trimedlure (Suterra LLC, Bend, OR).

2.3 Field Cage Bioassays

Choice test bioassays of long-range attraction were conducted with sterile *C. capitata* in a mesh field cage (3.8 m wide x 13.9 m long x 3 m tall) at SHRS. Eight shepherd hooks (1.4 m high) were placed 1.8 m from a central release point at one end of the field cage and arranged in a circular pattern 45° from each other. Four potted foxtail palms, *Wodyetia bifurcate* (L.), non-host plants, were placed inside the field cage around the periphery of the shepherd hooks to provide resting sites for the flies. Lures consisted of a cotton wick (3.8 x 1.6 cm), either unbaited (control) or baited with a test substrate (10 µL per wick). For test 1, the wick was affixed to the center of a fluorescent, yellow sticky trap (7.6 x 15 cm). For test 2, the wick was placed in the basket in a Jackson trap (white delta trap, 9.5 cm^3 trap body, 9.5 cm by 12.7 cm sticky floor). The eight treatments consisted of traps that were unbaited or were baited with each of the six essential oils or trimedlure. Traps were hung on shepherd hooks and vented for 20 min before fly release. Position of the traps in the arena was randomized before each replicate. A rearing cage containing 500 flies was placed on a platform (30.5 cm above ground) at the center of the circle 15 min prior to release to allow flies to acclimate. After 4 hours, traps were sampled and number of flies per trap was recorded. There were eight replicates per test and at least 48 hours between replicates.

2.4 Field Tests

Long-range attraction of wild *C. capitata* was assessed through field tests conducted in two coffee farms (1280 m altitude), located 500 m apart, in Marcala, Departmento de La Paz, Honduras. Sites included the Mauricio Ramos Farm (14:09:24.18 N, 88:01:23.29 O) and the René Martínez Farm (14:09:23.79 N, 88:01:18.72 O). Both sites had citrus trees and non-host shade trees intercropped with the coffee trees. The experimental design was randomized complete block with 10 replicate blocks. At each site, standard 5 x 8 trapping grids were installed in coffee trees, with the traps placed in 5 alternating rows (blocks) that were 12 m apart. To prevent edge effects, no traps were placed closer than 20 m from the edge of the coffee field. The eight treatments consisted of Jackson traps that were unbaited or were baited with 1 ml of each of the six essential oils or trimedlure. Traps were sampled weekly for 4 weeks, and treatments were moved sequentially within a row after each sampling. Sum total number of males and females captured over the 4 week sampling period per block was used for subsequent analysis.

2.5 Laboratory Bioassays

Small cage bioassays were used to quantify the short-range attraction of sterile males to test substrates [20]. All tests were no-choice tests that were conducted between 1 pm and 5 pm at room temperature using cages containing flies from ~100 pupae. Dead flies and remaining pupae were removed prior to the start of a bioassay and the actual number of flies per cage available for tests was determined at the end of each bioassay. Preliminary tests found that < 0.05% of males were captured when offered untreated disks [20], so untreated controls were not used in the study. Test substrate (5 µL neat essential oil) was added to the center of a filter paper disk (Whatman #1, 5.5 cm diameter). The filter paper was placed into the bottom of a Petri dish (85 mm diameter, 12 mm height), which was then placed in the center of the cage with flies. After 5 minutes, the lid was placed on the dish, and the covered dish was removed from the cage. Percentage of flies responding, which was determined by dividing the number of flies in the dish by the sum total of the number flies in the dish plus the number of flies remaining in the cage at the end of each bioassay, was used for analysis. Flies and Petri dishes were used only once, and cages were pressure washed with hot water between tests to eliminate potential residual chemicals.

2.6 Electroantennography

EAG responses were recorded from fly antennae with a Syntech® system (Hilversum, The Netherlands) using methods described previously [20, 23]. Test substrates were prepared by placing each essential oil (3 ml) into a hermetic bottle (250 ml) fitted with septum port lid. Samples were allowed to equilibrate overnight at 24 °C to reach saturation. Freshly dissected antennal preparations were mounted between micropipette electrodes using conductive gel (Spectra 360; Parker Laboratories, Fairfield, NJ, USA) and placed under purified air flow (400 ml/min). Using gastight

syringes (VICI Precision Sampling, Baton Rouge, LA, USA), a sample (0.5 ml) of saturated vapor was withdrawn from each test bottle, injected into the airstream and presented to the antenna. In each recording session, the antenna was first presented with a standard reference sample, consisting of 100 µl 2-butanone saturated vapor (99% pure; Sigma-Aldrich, St. Louis, MO, USA). This was followed by injection of test samples (in random order), then by a negative control (0.5 ml clean air), and ended with a final injection of the standard. There was a 2 minutes interval of clean air flow between sample injections to prevent antennal adaptation. EAG responses were measured initially in millivolts (peak height of depolarization) and then normalized to percentages relative to the standard reference sample, using EAG 2000 software, which corrected for time dependent variability in antennal performance. Response to the negative control then was subtracted from the normalized test responses to remove any 'pressure shock' caused by injection volume. All statistical analyses were conducted using corrected normalized values. EAG responses were recorded from 15 insects, with each insect antenna exposed to all essential oils.

2.7 Chemical Collection and Analysis

Volatile chemicals were collected from essential oils using collector traps with 25 mg super-Q as the adsorbent (Analytical Research Systems, Inc., Gainesville, FL) using methods described previously [20, 24]. Volatile-collection traps were cleaned by soxhlet extraction using methylene chloride for 24 hours and dried in a fume hood prior to use. Essential oil (50 µl) was added to a cotton wick and placed immediately into a cylindrical glass chamber (4.5 cm diameter, 25 cm length). Purified air was introduced into the chamber using a push-pull collection system (1 L per min), and headspace volatiles were collected for 15 minutes. Chemicals were eluted from the super-Q adsorbent using 200 µl of high purity methylene chloride (99.5% pure; ACROS, Morris Plains, NJ). An aliquot of C_{16} standard (5 µg) was added to each sample for quantitative analysis.

Chemical extracts were analyzed by gas chromatography-mass spectroscopy (GC-MS). To identify the volatile chemicals, the following parameters were used for the Mass Spectrometer (Agilent Technologies 5975B): EI energy: 69.9 eV, MS source and MS quadrapole at 230 and 150 °C respectively, Electron Multiplier 1294 V. The column used in the gas chromatograph interface to the mass spectrometer was 25 m long, 0.25 mm I.D DB-5MS phase (J&W Scientific, Agilent Technologies) programmed at 40 °C for 2 min, then from 40-130 °C at 10.0 °C min^{-1}, then from 130-220 °C at 20.0 °C min^{-1}, and then held at 220 °C for 4 min. Chemicals were tentatively identified using the NIST Mass spectral program version 2.0d and NIST/EPA/NIH mass spectral library (NIST11) when Reverse Matches and Matches were > 950 and 900 ‰, respectively.

To verify chemical identifications generated by the mass spectral library, the RI values of sample peaks were compared with the RI values obtained from synthetic chemicals (when commercially available) or with previously published data obtained with comparable GC methods. Synthetic chemicals used for this analysis included of α-pinene (Aldrich Chemical Co., Milwaukee, WI), β-pinene (Aldrich Chemical Co.), D-limonene (Glidden Organics of SCM Corp., Jacksonville, FL), α-copaene (Fluka Analytical, Stenheim, Germany), (-)-linalool, (-)-terpinen-4-ol, β-myrcene, geraniol, β-caryophyllene, camphene and α-terpineol (Sigma Chemical Co., St. Louis, MO).

2.8 Statistical Analysis

Data were analyzed by analysis of variance (ANOVA, Proc GLM; SAS Institute 2010) followed by LSD mean separation (P = 0.05) for significant ANOVAs. When necessary, data were transformed prior to analysis to satisfy conditions of equal variance (Box *et al*. 1978), non-transformed means ± standard deviations are presented. One way ANOVAs were used to test effect of treatment. Pearson correlation tests (Statview 5.0.1, SAS Institute Inc.) were performed to compare chemical content with fly choices in experiments and with EAG responses.

3. Results

3.1 Long-range Attraction

Treatment affected number of flies captured in both the field cage bioassays and the field tests are given in table 1 and 2. In field cage bioassays, yellow sticky traps baited with trimedlure or ginger root oil were the most effective and caught more sterile males than the other baited traps or the unbaited control trap. Yellow sticky traps baited with orange oil or manuka oil also captured more sterile males than the unbaited traps. The remaining treatments (cubeb oil, angelica seed oil, tea tree oil) captured intermediate numbers of males, and there was no difference in capture with these oils versus unbaited traps or traps baited with orange oil or manuka oil. Captures with yellow sticky traps were correlated with the captures using Jackson traps (z = 2.449; P<0.05). Jackson traps baited with orange oil were as effective as those baited with trimedlure and ginger root oil, and all were more effective than Jackson traps baited with the remaining oils and the unbaited control. Tea tree oil had the lowest capture of all the test substrates, and there was no difference between traps baited with this oil or unbaited traps.

A similar pattern was observed for capture of wild males in the field test in Honduras (z = 4.355 and 2.155; P<0.0001 and P<0.05 for captures with yellow sticky traps and Jackson traps, respectively). However, in the field test, the highest capture was in trimedlure-baited Jackson traps, with intermediate capture in ginger root oil-baited Jackson traps, and there was no difference in capture in traps baited with the remaining oils or the unbaited control. Few females were captured in this study, but more were captured in ginger root oil-baited Jackson traps than in any other baited or unbaited Jackson trap.

3.2 Short-range Attraction

Males were captured in response to all substrates in the small cage bioassays (Table 3). The highest responses were elicited with angelica seed oil, tea tree oil and manuka oil. The lowest response was elicited by ginger root oil, and response to this oil was lower than to all other essential oils tested. These results were negatively correlated with the captures obtained in the field tests and in the field cage experiments (z = -2.384 and -2.497; P<0.05 respectively).

3.3 Electroantennography

Comparative EAG measurements (Fig. 1A) indicated that there were significant differences in amplitude of antennal response to fixed doses of vapor from the six natural essential oils (F = 5.188; P<0.01). EAG response was the highest for ginger root oil and the lowest for manuka oil, with intermediate response to all remaining oils. The amplitude of the antennal response was not correlated with the fly preferences in the field, long-range or short-range bioassays (z = 1.413, 1.274 and -1.329; P >0.05 respectively). However, if

manuka oil was excluded from the analysis, the antennal responses were positively correlated with the field and long-range bioassays (z = 3.48 and 3.99; P<0.001), and negatively correlated with the short-range bioassays (z = -1.985; P<0.05).

3.4 Chemical Analysis

GC-MS analysis identified 12 chemicals in common to most of the 6 natural essential oils, including 5 monoterpenes, 4 alcohols and 3 sesquiterpenes (Table 4). The emissions from ginger root oil consisted mainly of 4 monoterpenes: camphene, D-limonene, α-pinene and β-pinene (40, 27 and 15 and 14%, respectively), but also contained the highest concentrations of camphene, β-myrcene, β-pinene, linalool, α-terpineol and geraniol compared to the other oils analyzed. D-limonene was the predominant secondary metabolite quantified from orange and angelica seed oils, representing more than 98% of the total. α-Pinene was high in manuka oil (79%), and was present in high concentrations in combination with terpinen-4-ol and D-limonene in tea tree oil (16, 30, and 50%, respectively). α-Pinene, β-pinene and limonene were almost equally distributed in cubeb oil (34, 27, and 36%, respectively).

The amount of α-copaene was not correlated with attraction in the field test (z = -0.197, P>0.05), field cage experiments (z = -0.086 and -0.457; P>0.05 for sticky and Jackson traps respectively), nor short-range bioassays (z = 0.334; P>0.05). The amount of camphene, β-myrcene, β-pinene, linalool, α-terpineol and geraniol were all negatively correlated with fly preferences in the short-range bioassays (z = -2.185, -2.178, -2.115, -2.340, -2.451 and -2.178; P<0.05 respectively). However, all these chemicals (except β-pinene) were positively correlated with fly preferences in the field (z = 5.320, 5.320, 3.925, 2.973 and 5.320; P<0.01 respectively), and long-range bioassays using yellow sticky traps (z = 2.126, 2.145, 2.931, 3.823, 2.145; P<0.01) and using Jackson traps (z = 2.126, 2.145, 2.931, 3.823 and 2.145; P<0.05). Camphene, β-myrcene, linalool, α-terpineol and geraniol were all correlated with each other (P<0.01). EAG responses were slightly negatively correlated with the amount of α-copaene (z = -1.964; P<0.05; Fig. 1B), but this correlation was lost when manuka oil (which emitted a large quantity of α-copaene) was removed from analysis. However, EAG responses were positively correlated with the quantity of α-humulene emitted from the various essential oils (z=2.256; p<0.05).

Table 1: Number (mean ± std dev) of sterile male *C. capitata* per trap in choice tests conducted in a field cage. Traps were baited with 10 µl of each substrate and there were ~500 males released per replicate (n = 8)

Test substrate	Yellow sticky traps	Jackson traps
Trimedlure	87.3 ± 23.0a	69.8 ± 30.4a
Ginger root oil	71.4 ± 30.2a	54.3 ± 24.7a
Orange oil	23.5 ± 17.5b	37.6 ± 8.0a
Manuka oil	24.8 ± 14.8b	17.1 ± 7.8b
Cubeb oil	20.4 ± 13.5bc	16.3 ± 6.1b
Angelica seed oil	16.9 ± 11.1bc	17.5 ± 9.5b
Tea tree oil	10.9 ± 7.6bc	13.1 ± 10.5bc
Control (unbaited)	8.4 ± 10.8c	7.0 ± 5.9c
$F_{7,56}$	15.56	20.90
P	< 0.0001	< 0.0001

Means followed by the same letter within a column are not significantly different (P = 0.05, LSD mean separation test on log [x + 1]-transformed data, non-transformed means presented).

Table 2: Number (mean ± std dev) of *C. capitata* per trap over 4 wk in field tests conducted in Honduras in coffee. Traps were baited with 1 ml of each substrate (n = 10).

Test substrate	Males	Females
Trimedlure	37.3 ± 34.5a	0.2 ± 0.4b
Ginger root oil	13.1 ± 12.2b	2.7 ± 3.8a
Manuka oil	1.2 ± 2.3c	0.4 ± 0.7b
Cubeb oil	1.0 ± 1.9c	0.3 ± 0.7b
Orange oil	0.6 ± 1.0c	0.0 ± 0.0b
Angelica seed oil	0.1 ± 0.3c	0.2 ± 0.4b
Tea tree oil	0.0 ± 0.0c	0.0 ± 0.0b
Control (unbaited)	0.0 ± 0.0c	0.0 ± 0.0b
$F_{7,63}$	38.07	9.07
P	< 0.0001	< 0.0001

Means followed by the same letter within a column are not significantly different (P = 0.05, LSD mean separation test on log [x + 1]-transformed data, non-transformed means presented).

Table 3: Average percentage response (mean ± std dev) of sterile male *C. capitata* in small cage bioassays. Materials were presented as 5 µL samples of neat material. There were ~100 males per test substrate and replicate (n = 3).

Test substrate	Percent response
Angelica seed oil	36.5 ± 6.4a
Tea tree oil	33.5 ± 10.0ab
Manuka oil	29.8 ± 5.2abc
Orange oil	24.9 ± 4.7bc
Cubeb oil	21.0 ± 2.3c
Ginger root oil	6.9 ± 3.5d
$F_{5, 12}$	13.54
P	0.0001

Means followed by the same letter within a column are not significantly different (P = 0.05, LSD mean separation test on square root [x + 0.5]-transformed data, non-transformed means presented)

Table 4: Quantity (µg ± s.d.) of volatile chemicals emitted from 50 µl samples of essential oil. Volatiles collected with SuperQ adsorbant and analyzed by gas chromatography-mass spectroscopy; chemical identification confirmed by authentic standards on DB5 column

RI	Chemical	Ginger Root Oil	Orange Oil	Manuka Oil	Angelica Seed Oil	Cubeb Oil	Tea Tree Oil
943	α-pinene	167.3±37.2	11.2±7.0	165.6±74.4	42.1±13.3	170.0±58.0	105.9±4.8
959	camphene	427.6±94.3	0.1±0.0	0.9±0.2	4.4±1.4	6.0±1.1	0.9±0.6
988	β-myrcene	0.4±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
994	β-pinene	157.1±40.1	0.4±0.4	11.6±4.5	6.7±2.4	132.6±33.1	31.9±1.9
1039	D-limonene	299.7±93.9	1788±406	5.6±1.5	279±99.3	180.2±47.7	199.2±63.7
1103	linalool	26.1±5.1	5.7±1.7	1.9±0.4	0.5±0.6	0.4±0.4	0.0 ±0.0
1189	terpinen-4-ol	1.4±0.5	9.6±5.3	1.7±2.4	0.1±0.0	3.9±5.4	335.1±99.1
1199	α-terpineol	2.6±0.6	1.0±0.4	0.1±0.1	0.0±0.0	0.13±0.2	0.0±0.0
1256	geraniol	4.5±1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1398	α-copaene	0.9±0.2	0.2±0.1	17.9±6.1	0.5±0.2	0.5±0.1	0.9±0.4
1448	β-caryophyllene	0.0±0.0	0.0±0.0	4.8±1.5	0.1±0.1	0.0±0.0	0.9±0.2
1485	α-humulene	0.5±0.5	0.4±0.4	0.3±0.3	0.4±0.4	0.5±0.4	0.3±0.3

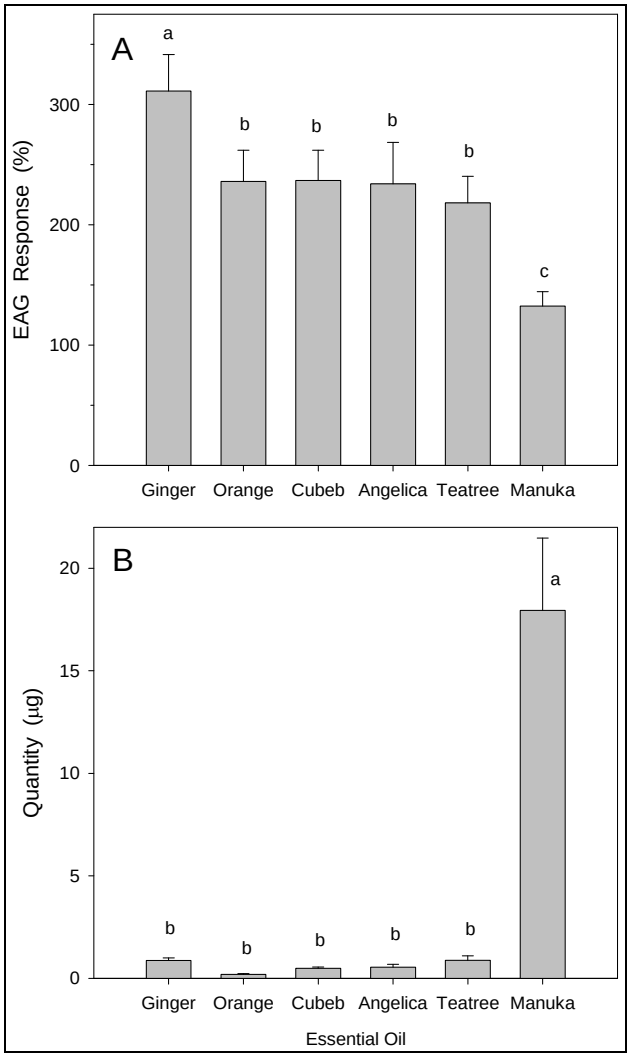

Fig 1: (A) Electroantennogram response (mean ± SE) of sterile male *C. capitata* to fixed doses (0.5 ml saturated vapor) of volatiles emitted from essential oils, given as a percentage relative to the standard reference compound (2-butanone, 100 µl saturated vapor). (B) Quantity (mean ± SE) of α-copaene emitted from essential oils, as determined by super-Q collections and GC-MS analysis. Bars topped with the same letter are not significantly different (ANOVA, LSD, $p<0.05$).

4. Discussion

The present study results, both in field tests and in field cage assays, found differential attractiveness among the essential oils tested, with higher long-range attraction to ginger root and orange oils compared to manuka, cubeb, and tea tree oils. Angelica seed oil, which was used for survey and detection during the 1956 *C. capitata* eradication program [11], was found to be much less attractive than orange oil or ginger root oil in our tests. Thus, the angelica seed oil used in our study is likely much different chemically than that used in previous studies, as angelica seed oil was found previously to be more attractive than cubeb oil using Jackson traps [19]. Published chemical analyses of essential oils (e.g. manuka oil, [25]) have shown that there can be much variability in terpene content, both qualitative and quantitative, among extracts obtained from trees in different geographic regions. In our field tests, female *C. capitata* were captured with ginger root oil; however, previous studies found no attraction of females to ginger root oil in field cage tests [7]. Again, this may be due to differences in the chemical constituents of the different sources of ginger root oil or to differences between laboratory females in field cage tests versus wild females in field tests.

Our electroantennographic analysis recorded the highest antennal response with ginger root oil (the most attractive oil in the field), the lowest response with manuka oil, and no significant differences among responses with the other oil substrates. With the exception of ginger root oil, the EAG responses did not follow the fly preferences observed in our long-range experiments. Since EAG measurements are the summation of potentials from olfactory receptors across the insect antenna, both volatile attractants and repellents elicit an EAG response, and it is not possible to identify the specific chemicals that contribute to that response. However, the antennal responses were positively correlated with the amount of α-humulene emitted from the essential oils. Niogret *et al.* [20] previously reported a correlation between *C. capitata* EAG response and the quantity of α-humulene emitted from wood substrates.

Previous studies have suggested that the sesquiterpene α-copaene was the primary compound responsible for attraction of male *C. capitata* to essential oils [18,19,26]. This chemical was identified in all the oils evaluated in this study. Despite its documented role in attraction of male *C. capitata*, the amount of α-copaene was not positively correlated with the oil's attractiveness in either field or laboratory experiments. For instance, manuka oil emitted the highest quantities of α-copaene (significantly more than ginger root oil), yet manuka was not very attractive to male *C. capitata* in the field. In addition, manuka oil elicited the lowest EAG response. This lack of correlation between α-copaene content and behavioral/electrophysiological response of *C. capitata* has been documented previously in comparisons of rasped bark and cambium from several tree species [20]. These results strongly suggest that other volatile chemicals may act synergistically with α-copaene in attraction of males.

Four known precursors of the male *C. capitata* pheromone were identified in the emissions from the tested essential oils: β-myrcene, limonene, linalool, and geraniol [27-29]. Kouloussis *et al*. [29] demonstrated that exposure to these precursors enhances male reproductive success. Therefore it is highly possible that attraction of *C. capitata* males to these chemicals is mediated by the resulting increase in mating success. For instance, male *Bactrocera dorsalis* (Hendel) are attracted to and feed on methyl eugenol, and benefit directly from this attraction by improvement of their mating success [15, 30]. By down-regulating the expression of a limonene synthase (introduction of an antisense construct) and consequently reducing the quantity of limonene accumulated in the oil glands of orange fruits, Rodriguez *et al*. [31] demonstrated a subsequent decrease in attraction of male *C. capitata*, suggesting that limonene plays a key role in attraction to orange fruit. However, interpretation of these results is not straightforward, since the process also resulted in reduction of other monoterpenenes and sesquiterpenes, which cannot be excluded as potential semiochemicals for *C. capitata*. In our experiments, limonene was not correlated with attraction of males, but emissions of linalool, β-myrcene and geraniol were positively correlated with fly preferences in both the field tests and field cages assays, which make them ideal candidates for future evaluations as *C. capitata* attractants. α-Terpineol and camphene are also chemicals of interest, since they too were positively correlated with attraction of males in the field.

The combined results of our field cage and field experiments identified five chemicals from essential oils as potential long-range attractants – linalool, camphene, α-terpineol, β-myrcene and geraniol. However, all five were negatively correlated with attraction of male *C. capitata* in short-range bioassays. This apparent contradiction may be the result of dose effects on behavioral response. It has been shown that male *C. capitata* exhibit arrestant behaviors and quiescence when in close proximity to essential oils containing α-copaene [3]. In general, the quantity of terpenoids emitted from essential oils exceeds the concentration found under natural conditions [3], and one (or more) of these chemical candidates may function as an arrestant or repellent at the higher doses encountered close to the source. Additional bioassays with serial dilutions of essential oils are needed to further address potential dose effects.

5. Conclusion

The present study demonstrated differential attractiveness of six plant essential oils to males of the Mediterranean fruit fly. Of the oils evaluated, ginger root oil was the most effective long-range attractant. Hierarchy of attraction was not correlated with the quantity of emitted α-copaene, the sesquiterpene traditionally accepted as a primary male attractant and cue for lek formation. Future research on attractants for *C. capitata* will focus on several terpenoid chemicals, including β-myrcene, linalool, geraniol, camphene, and α-terpineol, all of which were detected at higher levels in ginger root oil than in the other oils analyzed**.**

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