Insecticidal Activity of Basil Oil, *trans*-Anethole, Estragole, and Linalool to Adult Fruit Flies of *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae*

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ABSTRACT Basil oil and its three major active constituents (*trans*-anethole, estragole, and linalool) obtained from basil (*Oscimum basilicum* L.) were tested on three tephritid fruit fly species [*Ceratitis capitata* (Wiedemann), *Bactrocera dorsalis* (Hendel), and *Bactrocera cucurbitae* (Coquillett)] for insecticidal activity. All test chemicals acted fast and showed a steep dose–response relationship. The lethal times for 90% mortality/knockdown (LT_{90}) of the three fly species to 10% of the test chemicals were between 8 and 38 min. The toxic action of basil oil in *C. capitata* occurred significantly faster than in *B. cucurbitae* but slightly faster than in *B. dorsalis*. Estragole acted faster in *B. dorsalis* than in *C. capitata* and *B. cucurbitae*. Linalool action was faster in *B. dorsalis* and *C. capitata* than in *B. cucurbitae*. *trans*-Anethole action was similar to all three species. Methyl eugenol acted faster in *C. capitata* and *B. cucurbitae* than in *B. dorsalis*. When linalool was mixed with cuelure (attractant to *B. cucurbitae* male), its potency to the three fly species decreased as the concentration of cuelure increased. This was due to linalool hydrolysis catalyzed by acetic acid from cuelure degradation, which was confirmed by chemical analysis. When methyl eugenol (*B. dorsalis* male attractant) was mixed with basil oil, *trans*-anethole, estragole, or linalool, it did not affect the toxicity of basil oil and linalool to *B. dorsalis*, but it did significantly decrease the toxicity of *trans*-anethole and estragole. Structural similarity between methyl eugenol and *trans*-anethole and estragole suggests that methyl eugenol might act at a site similar to that of *trans*-anethole and estragole and serve as an antagonist if an action site exists. Methyl eugenol also may play a physiological role on the toxicity reduction.

KEY WORDS essential oil, *trans*-anethole, estragole, linalool, fruit fly

Basil belonging to the genus *Ocimum* (Lamiaceae) contains up to 150 species of herb and shrubs in the tropical regions of Asia, Africa, and Central and South America (Simon et al. 1990) and is a rich source of essential oils. Sweet basil (*Ocimum basilicum* L.), a common garden herb, is cultivated in the United States, Turkey, Yugoslavia, Iran, Thailand, Zambia, and Taiwan for culinary purposes as a fresh herb and as a dried spice. Basil has the innate ability to discourage herbivory when insects are not abundant; therefore, it can be used as an alternative to chemical pest control (Olson and Bidlack 1997). Basil oil, extracted via steam distillation from the leaves and other parts of the plants, is used to flavor foods, dental and oral products, and a fragrance in traditional rituals and medicines (Simon et al. 1990, Kirbaslar 2001). Basil oil contains bioactive constituents that are insecticidal (Deshpande and Tipnis 1977, Chogo and Crank 1981, Chavan and Nikam 1982, Keita et al. 2001, Salvatore et al. 2004), repellent (Maganga et al. 1996, Tawatsin et al. 2001, Paula et al. 2004, Popovic et al. 2006), nematicidal (Chatterje et al. 1982), fungistatic (Reuveni et al. 1984), or antimicrobial (Nitezurubanza et al. 1984). These properties can frequently be attributed to predominant essential oil constituents such as methyl chavicol (estragole), methyl eugenol, linalool, camphor, and methyl cinnamate (Baritaux et al. 1992).

Pest tephritid fruit flies directly damage crops and are quarantine species. Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann); oriental fruit fly, *Bactrocera dorsalis* (Hendel); and melon fly, *Bactrocera cucurbitae* (Cocquillett) are three species of tephritid fruit flies of current economic significance in Hawaii and worldwide. Control and detection of these fly species have primarily relied on the use of food baits, parapheromones (i.e., male attractants), and their combinations with insecticides. Medlure, methyl eugenol, and cuelure are known to be male attractants to Mediterranean, oriental, and melon flies, respectively (Metcalf et al. 1975, Vargas et al. 2000). To our knowledge, insecticidal activities of basil oil to tephritid fruit flies and interactions of basil oil or its constituents with male fruit fly attractants on fruit fly have not been reported. The objectives of this study were to inves-

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Table 1. Mean mortality/knockdown of *C. capitata*, *B. dorsalis*, and *B. cucurbitae* in response to various concentrations (0–100%) of basil oil, *trans*-anethole, estragole, linalool, or methyl eugenol for at least 2 h

% chemical[a]	Mortality/knockdown (%) ± SE[b]				
	Basil oil	*Trans*-anethole	Estragole	Linalool	Methyl eugenol
C. capitata					
0	0	0	0	0	0
0.25	0	0	0	0	31 ± 9
0.5	0	0	0	0	96 ± 5
0.75	0	0	0	0	100
1	0	75 ± 4	61 ± 5	0	100
2.5[c]	95 ± 4	100	100	90 ± 5	100
B. dorsalis					
0	0	0	0	0	0
1	68 ± 11	92 ± 11	1 ± 9	5 ± 2	3 ± 4
2.5	77 ± 13	100	100	100	0
5	85 ± 9	97 ± 5	100	100	0
7.5	87 ± 13	88 ± 13	100	100	0
10	100	90	100	100	35 ± 18
25	100	100	100	100	38 ± 17
50	100	100	100	100	41 ± 8
100	100	100	100	100	52 ± 13
B. cucurbitae					
0	0	0	0	0	0
0.25	0	0	0	0	0
0.5	0	0	0	0	84 ± 5
0.75	0	0	0	0	98 ± 6
1	0	98 ± 4	51 ± 10	0	97 ± 9
2.5	93 ± 7	100	100	0	100
	100	100	100	90 ± 7	100
7.5	100	100	100	98 ± 9	100
10	100	97 ± 9	100	98 ± 5	100

[a] No mortality/knockdown to all the three fruit fly species was observed with 0.1% basil oil, *trans*-anethole, estragole, linalool, methyl eugenol, except for 1% mortality/knockdown caused by 0.1% methyl eugenol.
[b] No SE values are indicated when no or 100% mortality/knockdown of all replicates occurred.
[c] Basil oil, *trans*-anethole, estragole, linalool, and methyl eugenol (at 5, 7.5, or 10%) gave 100% mortality/knockdown to *C. capitata*.

tigate the insecticidal activities of basil oil and its major constituents, identify the lethal doses and to investigate the compatibility with methyl eugenol or cuelure for potential applications to "attract and kill" the fruit flies.

Materials and Methods

Insects. The three fruit fly species tested in this study are *C. capitata*, *B. dorsalis*, and *B. cucurbitae.* Pupae were provided by the fruit fly rearing facility in the Pacific Basin Agricultural Research Center of the United States Department of Agriculture, Agricultural Research Service (USDA–ARS) in Manoa, HI. All flies were 360 generations from the field and reared at 25°C, 65% of relative humidity (RH), and a photoperiod of 12:12 (L:D) h.

Chemicals. Basil oil (100% purity) was purchased from Dragonmarsh (Riverside, CA). Linalool was purchased from ICN Pharmaceuticals (Bryan, OH). Estragole (methyl chavicol) and *trans*-anethole were purchased from Sigma-Aldrich (St. Louis, MO). Cuelure and methyl eugenol were provided by Dr. Grant McQuate (USDA–ARS, Hilo, HI). All organic solvents (HPLC grade) and acetic acid (99% purity) were purchased from Fisher Scientific (Pittsburgh, PA).

Insecticidal Activity of Basil Oil and its Major Constituents. Pure basil oil was diluted to 0.1, 1, and 10% by volume in acetone and used for preliminary mortality/knockdown tests (see description of bioassays below) on *C. capitata*, *B. dorsalis*, and *B. cucurbitae.* Acetone and chloroform were used as carrier controls. Complete mortality/knockdown was observed with 10 and 100% basil oil, whereas 0.1% basil oil showed no mortality/knockdown in any of the three fruit fly species (Table 1). To elucidate bioactive constituents in the basil oil, the basil oil was analyzed on a gas chromatograph-mass spectrometer (GC/MS) for component profiles. The three major constituents, *trans*-anethole, estragole, and linalool, were selected for insecticidal activity tests at varying concentrations based on their relative concentrations in basil oil. Although methyl eugenol is a minor component in basil oil, it was selected for insecticidal activity tests because its structure is very similar to those of *trans*-anethole and estragole.

Bioassay. One-half milliliter of each test concentration solution was applied to one end of a 4-cm dental roll with a treatment label. The roll was placed in a chemical hood to evaporate acetone for 30 min before test. Twenty adult fruit flies (either males or females or both depending on bioassays) were introduced into a 500-ml distilled water bottle (Kirkland Signature, Niagara Bottling, Ontario, CA). The bottle was placed upright with the bottom side up and the cap side down. A 1-cm-diameter hole along with 100 pin holes for ventilation was made on the bottom of the bottle. A dental roll impregnated with the test chemical (e.g., basil oil, *trans*-anethole, estragole, or linalool) was inserted into the 1-cm hole. No direct contact of the flies with the dental roll was observed. Insects that fell onto the cap of the bottle after they responded to the chemical were considered dead and were counted. The lethal time giving 90% mortality/knockdown (LT_{90}) was observed and recorded. The chemical-impregnated wicks were removed from the bottles with 100% mortality/knockdown and replaced with a clean wick to observe whether the insects would recover. Three batches of insects (separate experiments) were used for each treatment. Four replicates of each batch were tested. Numbers of knockout/dead flies were recorded every 15 min for two to four consecutive hours from fly release depending on different outcomes.

Chemical Analysis and Identification. Constituents in basil oil were separated and identified using gas chromatography with flame ionization detection and a GC-MS. GC-MS analysis was performed on a Varian CP-3800 gas chromatograph-Saturn-2000 mass spectrometer (Varian, Inc., Walnut Creek, CA) equipped with a ZB-1 column (60 m, 0.25-μm film thickness; Phenomenex, Inc., Torrance, CA). Helium was the carrier gas at a flow rate of 2 ml/min. The column temperature started from 70°C (3 min), rose to 250°C at a rate of 4°C/min and then was held at 250°C for 10 min. The injector and transfer line temperatures were 250 and 280°C, respectively. The mass spectrometer was operated in electron impact mode at 70 eV. GC-flame ionization detector (FID) analysis was performed on a Hewlett–Packard 5890 gas chromatograph equipped with a DB-35MS (15 m $\times$ 0.2 mm $\times$ 0.33 μm film thickness, J&W Scientific, Folsom, CA). The column temperature started from 50°C (2 min), rose to 250°C at a rate of 25°C/min, and was held at 250°C for 5 min. The injector and analyzer temperatures were 150 and 250°C, respectively.

Insecticidal Activity of Basil Oil and its Constituents Mixed with Cuelure and Methyl Eugenol. Estragole, *trans*-anethole, and linalool were selected for further tests in a mixture with cuelure or methyl eugenol. Mixtures of two chemicals were made with 10% linalool and varying concentrations of cuelure (1, 10, 25, 50, and 90% for studies of effects of cuelure on mortality/knockdown) or 10% *trans*-anethole, estragole, or linalool and 90% methyl eugenol. Controls treated in the same manner were 10% *trans*-anethole, estragole, or linalool alone and appropriate concentrations of cuelure or methyl eugenol alone. Acetone and chloroform were solvent controls. Potency of chemical mixtures was tested by following the same procedures as those for individual chemicals. Numbers of knockdown/dead flies were recorded every 15 min for two to four consecutive hours depending on the outcomes.

Degradation of Linalool and *trans*-Anethole in Presence of Cuelure, Methyl Eugenol, or Acetic Acid. The mixtures of 10% linalool or *trans*-anethole and varying concentrations of cuelure (1, 10, 25, 50, and 90%) and 10% linalool or *trans*-anethole and 90% methyl eugenol were analyzed on GC-FID and GC-MS to monitor possible degradation of linalool or *trans*-anethole. A solution of linalool or *trans*-anethole (10%) in acetone was used as a control. Acetic acid (10%) was added to pure linalool (200 μl of linalool and 20 μl of acetic acid) to confirm whether cuelure causes the degradation of linalool. It is known that cuelure is unstable and can be easily hydrolyzed to give raspberry ketone and acetic acid (Alcantara-Licudine et al. 1996); the latter may catalyze degradation of linalool. After the solution was gently shaken for 30 s, an aliquot was sampled for GC-FID analysis at intervals of 1, 2.5, 5, and 10 h at ambient temperature.

Data Analysis. Insect mortality/knockdown and chemical degradation results were calculated for means and SEs. LT_{90} values were tested using an analysis of variance (ANOVA) (Sigma Stat, version 3.4, Systat Software, Inc., San Jose, CA).

Results and Discussion

Identification of Main Components in Basil Oil. In an effort to search for natural products to control tephritid fruit flies, we have found that basil oil is very potent to *C. capitata*, *B. dorsalis*, and *B. cucurbitae.* Our first goal was to identify the major components in basil oil. As shown in Fig. 1, 12 volatile components were identified on GC-MS by comparison of the MS spectra with those in NIST mass spectra database. These chemicals include α-pinene (1; $C_{10}H_{16}$), linalool (2; $C_{10}H_{18}O$), *trans*-anethole (3, $C_{10}H_{12}O$), 4-methoxy benzaldehyde (4; $C_8H_8O_2$), estragole (5; $C_{10}H_{12}O$), 1-methoxy-4-(1-methoxypropyl)-benzene (6; $C_{11}H_{16}$ O), *trans*-caryophyllene (7; $C_{15}H_{24}$), methyl eugenol (8; $C_{11}H_{14}O_2$), 3,7,11-trimethyl-(*E*)-1,6-dodecatriene-3-ol (9; $C_{15}H_{26}O$), 2,3-dihydro-1*H*-lndene-5-ol (10; $C_9H_{19}O$), 3-[3-iodo-2-(iodomethyl)-2-methylpropyl]-1,2,4,5-tetramethyl benzene (11; $C_{15}H_{22}I_2$), and 1-(1,1-dimethyl)-2-methoxy-4-methyl-3,5-dinitrobenzene (12; $C_{12}H_{16}N_2O_5$). Linalool (2), *trans*-anethole (3), and estragole (5) are major volatile components in basil oil. Estragole, *trans*-anethole, methyl eugenol, and linalool were further identified with GC-MS in comparison with their corresponding authentic standards. Estragole, methyl eugenol and linalool were reported to be the main components in the basil oil (Hink et al. 1988, Baritaux et al. 1992, Marotti et al. 1996, Consuelo et al. 2002). Estragole is a double-bond isomer of *trans*-anethole. Methyl eugenol has an extra methoxy group on the estragole molecule. Linalool is an antennally active compound to house flies, *Musca domestica* (L.) (Salvatore et al. 2004).

Insecticidal Activities of Basil Oil and its Constituents. Varying concentrations of basil oil, *trans*-anethole, estragole, linalool, and methyl eugenol were tested for mortality/knockdown on the three fruit fly species (Table 1). The five tested chemicals (basil oil is referred to as a chemical here) at 2.5% or higher, except linalool, produced >77% mortality/knockdown in all three fruit fly species after 2-h exposure. In general, *trans*-anethole and estragole were more potent than basil oil and linalool. It is interesting that methyl eugenol was very toxic to *C. capitata* and *B. cucurbitae*, but less toxic to *B. dorsalis* to which methyl

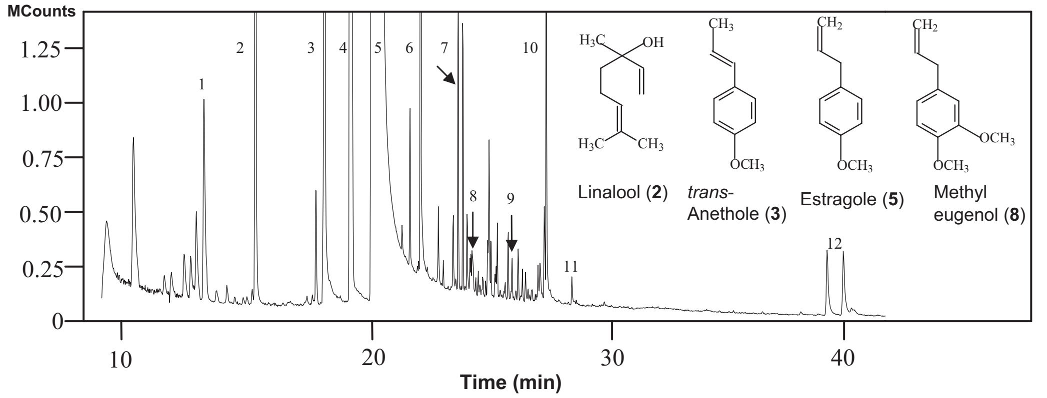

Fig. 1. Total ion current chromatograms of GC-Ion Trap MS for volatile components of basil oil (1000 μg/ml in acetone). Peak identification: 1: α-pinene ($C_{10}H_{16}$); 2: linalool [4-methyl-1-(1-methylethyl)-3-cyclohexan-1-ol, $C_{10}H_{18}O$, MW 154]; 3: *trans*-anethole [1-methoxy-4-(1-propenyl) benzene, $C_{10}H_{12}O$, MW 148]; 4: 4-methoxy benzaldehyde ($C_8H_8O_2$); 5: estragol [1-methoxy-4-(2-propenyl) benzene, $C_{10}H_{12}O$, MW 148]; 6: 1-methoxy-4-(1-methoxypropyl)-benzene ($C_{11}H_{16}O$); 7: *trans*-caryophyllene ($C_{15}H_{24}$); 8: methyl eugenol ($C_{11}H_{14}O_2$); 9: 3,7,11-trimethyl-(*E*)-1,6-dodecatriene-3-ol ($C_{15}H_{26}O$); 10: 2,3-dihydro-1*H*-indene-5-ol ($C_9H_{19}O$); 11: 3-[3-iodo-2-(iodomethyl)-2-methylpropyl]-1,2,4,5-tetramethyl benzene ($C_{15}H_{22}I_2$); 12: 1-(1,1-dimethyl)-2-methoxy-4-methyl-3,5-dinitrobenzene ($C_{12}H_{16}N_2O_5$).

eugenol is a male lure. A common feature of these chemicals is a sharp dose–response relationship that makes determination of LC_{50} values difficult. LC_{50} values of methyl eugenol, *trans*-anethole, estragole, linalool, and basil oil to *C. capitata* were ≈0.25–0.5, 0.75–1, 0.75–1, 1–2.5, and 1–2.5%, respectively, within 2-h exposure time. LC_{50} values of *trans*-anethole, estragole, linalool, and basil oil to *B. dorsalis* were ≈0.1–1, 1–2.5, 1–2.5, and 0.1–1%, respectively. LC_{50} values of methyl eugenol, *trans*-anethole, estragole, linalool, and basil oil to *B. cucurbitae* were ≈0.25–0.5, 0.75–1, 1, 2.5–5, and 1–2.5%, respectively. Approximate LC_{90} values of *trans*-anethole and estragole (except estragole to *B. dorsalis*) were less than those of basil oil and linalool (Table 1).

Fast action is another unique feature of *trans*-anethole, estragole, linalool, and basil oil to the three fly species. Table 2 shows similar LT_{90} values of *C. capitata* among *trans*-anethole (17 min), estragole (15 min), linalool (15 min), and basil oil (17 min). The LT_{90} value of estragole to *B. cucurbitae* (15 min) was less than those of *trans*-anethole (29 min), linalool (38 min), and basil oil (32 min). Estragole and linalool had a faster insecticidal action toward *B. dorsalis* than *trans*-anethole and basil oil. In general, both *C. capitata* and *B. dorsalis* were more susceptible to *trans*-anethole, estragole, linalool, and basil oil than *B. cucurbitae.* The insects knocked down were not able to recover 4 h after exposure to these chemicals. However, when the wicks were removed 2 h after exposure, some insects gradually recovered. The percentages of the dead/knockout flies after 2-h exposure to 10% linalool that were able to recover were 35 ± 5 and 26 ± 8 for female and male *B. cucurbitae*, respectively; 5 ± 3 and 18 ± 9 for female and male *B. dorsalis*, respectively; and 0 for both female and male *C. capitata.* However, all three species exposed to 2.5% estragole for 2 h were unable to recover after removal of the wicks.

Basil Oil, *trans*-Anethole, or Linalool Mixed with the Male Attractant Cuelure. Basil oil, *trans*-anethole, or linalool was mixed with varying concentrations of

Table 2. Mean lethal times for 90% mortality/knockdown[a] of 3-d-old male and female *C. capitata*, *B. dorsalis*, and *B. cucurbitae* under exposure for 4 h to 10% basil oil, *trans*-anethole, estragole, or linalool

Chemical	LT_{90} (min) ± SE			Statistics
	C. capitata	*B. dorsalis*	*B. cucurbitae*	
Trans-anethole	17 ± 1aA	26 ± 4aA	29 ± 4aA	$F = 3.51$; df = 2, 11; $P = 0.0749$
Estragole	15 ± 0aA	8 ± 0bB	15 ± 0aB	F = Infty; df = 2, 11; $P < 0.0001$
Linalool	15 ± 1bA	11 ± 1bB	38 ± 1aA	$F = 152.97$; df = 2, 11; $P < 0.0001$
Basil oil	17 ± 1bA	26 ± 5abA	32 ± 1aA	$F = 6.16$; df = 2, 11; $P = 0.0206$
Statistics	$F = 2.10$ df = 3, 15 $P = 0.1539$	$F = 12.27$ df = 3, 15 $P = 0.0006$	$F = 22.19$ df = 3, 15 $P < 0.0001$	

Within a column, means followed by the same letter (uppercase) are not significantly different ($\alpha = 0.05$; PROC ANOVA, Tukey test). Within a row, means followed by the same letter (lowercase) are not significantly different.

[a] The dead flies were not recovered after 4 h of exposure. Some of the knockout flies on the bottom of the battle exposed to the chemicals for <2 h could recover, and thus the knockout flies were considered as mortality/knockdown.

Table 3. Comparison of mean mortality/knockdown from three species of 3-d-old (younger) and 10-to 12-d-old (mature) adult fruit flies exposed to linalool, cuelure, and their combinations for 2 h

Treatment[a]	Mean mortality/knockdown (%) ± SE[b]											
	B. cucurbitae				*B. dorsalis*				*C. capitata*			
	3 d old		12 d old		3 d old		11 d old		3 d old		10 d old	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Chloroform	0	0	0	0	0	0	0	0	0	0	0	0
Acetone	0	0	0	0	0	0	0	1 ± 1	0	0	4 ± 2	4 ± 1
10 LL	100	100	100	100	100	100	100	100	100	100	100	100
10 LL+1 CL	100	100	100	100	100	100	100	100	100	100	100	100
10 LL+10 CL	98 ± 1	98 ± 1	71 ± 11	100	100	100	100	100	100	100	100	100
10 LL+25 CL	39 ± 6	45 ± 16	16 ± 5	55 ± 4	100	95 ± 2	100	98 ± 3	100	100	100	100
10 LL+50 CL	4 ± 2	6 ± 2	0	0	95 ± 2	0	38 ± 19	20 ± 11	100	100	100	100
10 LL+90 CL	5 ± 4	3 ± 1	0	0	3 ± 3	0	0	0	65 ± 6	100	3 ± 1	100
100 CL	1 ± 1	1 ± 1	0	0.3 ± 0.3	0	1 ± 1	0	0	0	0	1 ± 1	0

[a] LL and CL stand for linalool and cuelure, respectively. The numbers indicate percentages in the mixtures.
[b] No standard error values are indicated when no or 100% mortality/knockdown of all replicates occurred.

cuelure (a male attractant to *B. cucurbitae*) to develop an attract and kill formulation for fruit fly management.

Basil Oil and Cuelure. When 3- or 12-d-old *B. cucurbitae* males and females together (1:1 ratio) were exposed to 10% basil oil that was mixed in 1, 10, 25, 50, and 90% of cuelure for 2 h, the mean mortality/knockdown was 100 ± 0, 100 ± 0, 96 ± 2, 18 ± 9, and 10 ± 8%, respectively. Cuelure at high concentrations (50 and 90%) in a mixture with basil oil notably reduced the mortality/knockdown in *B. cucurbitae* males and females. Therefore, the two major basil oil constituents, *trans*-anethole and linalool, were tested for the effects of cuelure on their potency to the fruit flies.

trans-*Anethole and Cuelure.* When 3- or 12-d-old *B. cucurbitae* males and females together (1:1 ratio) were exposed to 10% *trans*-anethole in the presence of 1, 10, 25, 50, and 90% of cuelure for 2 h, the mean mortality/knockdown was 100 ± 0, 100 ± 0, 99 ± 1, 94 ± 2, and 0 ± 0%, respectively. Cuelure at 90% in a mixture eliminated the potency of *trans*-anethole to *B. cucurbitae* males and females at the test conditions.

Linalool and Cuelure. Table 3 shows the effects of cuelure on reducing mortality/knockdown of immature (3-d-old) and mature (10–12-d-old) fruit fly adults of the three species exposed to linalool for 2 h. Both linaool alone (10%) and a mixture of 10% linalool and 1% cuelure caused 100% mortalities (Table 3). Cuelure is an attractant to *B. cucurbitae* males. However, the survival rates increased as the concentrations of cuelure increased in the mixture with linalool. For example, the survival of both 3-d-old *B. cucurbitae* males and females gradually increased as concentration of cuelure in the mixture increased from 10 to 90%. For 12-d-old *B. cucurbitae*, the mortality/knockdown of the females started to decrease after addition of 10% cuelure and the males after addition of 25% cuelure. The males seemed to exhibit greater sensitivity than the females. For *B. dorsalis*, mortality/knockdown was eliminated by addition of 90% of cuelure for both sexes of either age. With *C. capitata,* addition of up to 50% cuelure did not affect mortality/knockdown for either 3- and 10-d-old male or female flies. There was no mortality/knockdown for 10-d-old *C. capitata* females with 90% cuelure, but 65% mortality/knockdown was observed for the 3-d-old female flies. Neither acetone nor chloroform, the carrier, showed any toxicity to the three fruit fly species. Because cuelure is a parapheromone, its effect on reducing the toxicity of basil oil, *trans*-anethole and linalool also could be due to physiological effects. Cuelure is known to be unstable and readily hydrolyzed into raspberry ketone and acetic acid (Alcantara-Licudine et al. 1996) that in turn could catalyze degradation of *trans*-anethole, linalool, and basil oil components, reducing their potency. This hypothesis was supported by disappearance of linalool in the presence of acetic acid as described in the next section. Raspberry ketone derived from cuelure was detected, although no specific reaction products between linalool and raspberry ketone or acetic acid were identified.

Basil Oil, Linalool, or *trans*-Anethole Mixed with Methyl Eugenol. Methyl eugenol (a male attractant to *B. dorsalis*) was tested to see whether it reduces the toxicity of basil constituents. *B. dorsalis* 3-d-old adults were exposed to a mixture of 10% basil oil, *trans*-anethole, estragole, or linalool and 90% methyl eugenol. Methyl eugenol did not reduce the mean mortality/knockdown from exposure to basil oil (100 versus 99%) or linalool (100 versus 100%), but it did dramatically decrease the mortality/knockdown from exposure to *trans*-anethole (98 ± 1 versus 19 ± 17%). Whereas 2.5% estragole caused 100% mortality/knockdown (Tables 1 and 4) and 50% methyl eugenol caused 41% mortality/knockdown (Table 1), the mixture of the two caused no mortality/knockdown in *B. dorsalis* (Table 4). The same phenomenon also was observed for the mixture of 10% *trans*-anethole and 90% methyl eugenol (Table 4). Methyl eugenol (90%) did not reduce the toxicity when the concentration of estragole was 10%. It should be noted that methyl eugenol is structurally very similar to *trans*-anethole and estragole, but very different from linalool. GC-MS analysis of the mixture of *trans*-anethole-methyl eugenol showed that methyl eugenol did not react with

Table 4. Mortality/knockdown of 3-d-old *B. dorsalis* after exposure (1 h) on 10% basil oil, linalool, *trans*-anethole, or estragole mixed with 90% methyl eugenol and exposure to 2.5% estragole mixed with 50% methyl eugenol

Treatment	Mortality/knockdown (%) ± SE[a]
10% basil	100
10% basil oil + 90% methyl eugenol	99 ± 1
10% linalool	100
10% linalool + 90% methyl eugenol	100
10% *trans*-anethole	98 ± 1
10% *trans*-anethole + 90% methyl eugenol	19 ± 17
10% estragole	100
10% estragole + 90% methyl eugenol	100
2.5% estragole	100
2.5% estragole + 50% methyl eugenol	0

[a] No SEs are indicated when no or 100% mortality/knockdown occurred. When *B. dorsalis* exposed to methyl eugenol for 1 h, no flies fell on the cap of the test bottles (bottles placed upside down), which is no mortality, for all concentrations. The mortality/knockdown occurred when the methyl eugenol exposure time was over 2 h as shown in Table 1.

trans-anethole or catalyze *trans*-anethole degradation within 3 h. It is possible that methyl eugenol might share a similar action site(s) as *trans*-anethole and estragole, but with less affinity for site than *trans*-anethole or estragole. Methyl eugenol also may play a physiological role on the toxicity reduction. This phenomenon warrants further investigation.

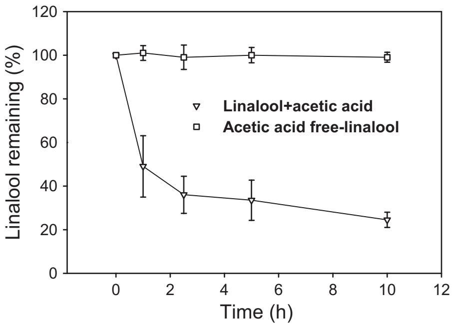

Fig. 3. Degradation kinetic of linalool in presence of acetic acid (20 μl of acetic acid in 200 μl of linalool). Acetic acid-free linalool was the control.

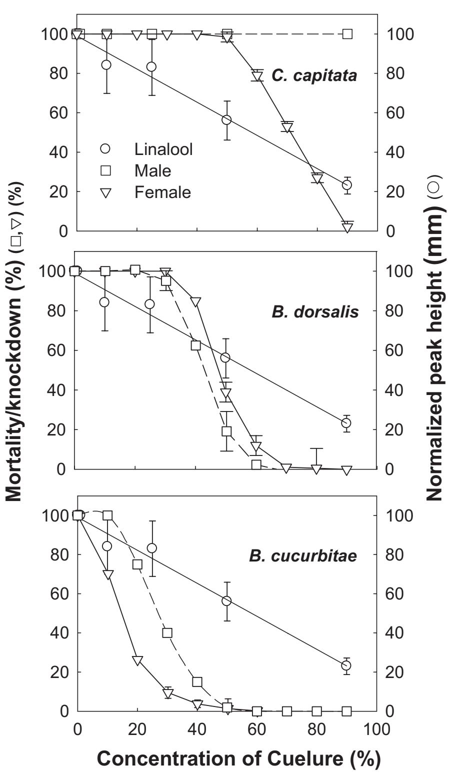

Fig. 2. Inverse relationship between mean mortality/knockdown of 11-d-old male (□) and female (▽) flies of the three species in response to varying concentrations of cuelure in 10% linalool, and decrease of normalized GC peak areas of linalool (○) in the linalool–cuelure mixtures as a function of cuelure concentration changes.

Cuelure Causes Degradation of Linalool or *trans*-Anethole and Reduces its Insecticidal Activities. It is interesting that cuelure reduced the potency of linalool to *C. capitata, B. dorsalis,* and *B. cucurbitae* (Fig. 2). Cuelure can be readily hydrolyzed into raspberry ketone and acetic acid and the latter may catalyze linalool degradation and thus reduces its potency. Therefore, mixtures of linalool (10%) or *trans*-anethole and varying concentrations of cuelure were analyzed for degradation products. The peak area of linalool decreased when the concentration of cuelure in the mixture increased. Raspberry ketone was constantly detected, which indicates hydrolysis of cuelure into raspberry ketone and acetic acid. Linalool is a tertiary alcohol and can be easily degraded in acidic conditions. Therefore, degradation of linalool in the presence of acetic acid was monitored (Fig. 3). More than 50% of the linalool was degraded within 1 h, which explains the decreasing mortality/knockdown from linalool as cuelure concentrations increased. GC-MS analysis showed that methyl eugenol does not facilitate linalool degradation (data not shown).

In conclusion, *trans*-anethole, estragol, and linalool are major bioactive compounds in basil oil against the tephritid fruit flies. Methyl eugenol is a minor component in basil oil, but it is very potent to *C. capitata* and *B. cucurbitae.* The action of these chemicals is fast and shows a steep dose–response relationship. It is interesting that methyl eugenol shares structural similarities to *trans*-anethole and estragole and reduces their potency to *B. dorsalis,* suggesting that methyl eugenol might act at a site similar to that of *trans*-anethole and estragole and serve as an antagonist. Methyl eugenol also may play a physiological role on the toxicity reduction. If *trans*-anethole, estragole, linalool, methyl eugenol, and basil oil are mixed with attractants such as paraphermones in an appropriate formula, they may be used as a natural insecticide.

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