MATING SUCCESS OF MALE MEDITERRANEAN FRUIT FLIES FOLLOWING EXPOSURE TO TWO SOURCES OF α-COPAENE, MANUKA OIL AND MANGO

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ABSTRACT

Recent studies on the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wied.), have demonstrated an increase in male mating competitiveness following exposure to particular plant structures or products, including the fruit and fruit-derived oil of orange trees, the bark and fruits of guava trees, and ginger root oil. Although it is not known which compound(s) was responsible for the enhanced mating success, all the performance-boosting substances tested thus far contain the sesquiterpene hydrocarbon α-copaene, and α-copaene tested alone was found to increase mating success in male medflies. As the concentration of α-copaene and other terpenes vary among plant species, it is not known whether exposure to α-copaene-bearing plants (or their derived oils) will universally influence the mating behavior of male medflies. The goal of this study was to describe the results of mating trials conducted after male exposure to 2 previously untested sources of α-copaene, manuka oil (from the New Zealand manuka tree) and mango fruits from cultivars in Hawaii and Guatemala. Mating trials conducted in field-cages revealed that exposure to manuka oil significantly increased the mating success of both wild males and mass-reared, sterile males. However, exposure to mangos had no effect on male mating performance in trials run in Hawaii or Guatemala. This latter result may have reflected the absence (or presence in very small amounts) of α-copaene in the mango cultivars tested or a particular mixture of compounds that diminished or blocked α-copaene's effect on the male medflies.

Key Words: *Ceratitis capitata*, Tephritidae, manuka oil, mango, mating behavior

RESUMEN

Estudios actuales sobre la mosca mediterránea del fruto, *Ceratitis capitata* (Wied.), han demonstrado un aumento en la capacidad de los machos para competir en el apareamiento después de ser expuestos a estructuras o productos de plantas particulares, incluyendo el fruto o aceite derivado del fruto, la corteza y el fruto del árbol de guajava, y aceite de la raíz del jengibre. Aunque no se sabe cual de estos químicos compuestos fue responsable para mejorar el éxito del apareamiento, todas las substancias probadas hasta ahora que aumentan la capacidad para ejecutar el apareamiento contienen el hidrocarburo sesquiterpene α-copaene, y se encontró que las pruebas con solo α-copaene resultaron en un aumento en el exito de apareamiento de los machos de la mosca mediterránea. Como la concentración de α-copaene y otros terpenes varia en las diferentes especies de plantas, no se sabe si la exposición a plantas que contienen α-copaene (o el aceite derivado de ellas) influye universalmente el comportamiento de apareamiento de los machos de la mosca mediterránea. La meta de este estudio fue para describir los resultados de las pruebas del apareamiento realizadas después de exponer los machos a 2 fuentes de α-copaene antes no probadas, el aceite de manuka (del arbol manuka de Nueva Zelanda) y el fruto de variedades de mango de Hawaii y Guatemala. Las pruebas de apareamiento realizadas en jaulas en el campo mostraran que la exposición al aceite de manuka aumento significativamente el éxito de apareameinto de machos esteriles tanto salvajes como criados en masa. Sin embargo, la exposición al mango no tuvo ningún efecto sobre la ejecución del apareamiento de los machos en pruebas realizadas en Hawaii o Guatemala. Este último resultado puede reflejar la ausencia (o presencia de muy poca cantidad) de α-copaene en las variedades de mango probadas o en la mezcla específica de los químicos compuestos que diminyó o bloqueo el efecto de α-copaene's sobre los machos de la mosca mediterránea.

Recent studies on the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wied.), have demonstrated an increase in male mating competitiveness following exposure to particular plant structures or products, including (i) the fruit and fruit-derived oil of orange trees (*Citrus sinensis* L) (Papadopoulous et al. 2001, 2006; Shelly et al. 2004a), (ii) the bark and fruits of guava trees (*Psidium guajava* L) (Shelly & Villalobos 2004), and (iii) ginger root oil (*Zingiber officinale* Roscoe) (Shelly 2001). Although it is not known which compound(s) is responsible for the enhanced mating success, all the performance-boosting substances tested thus far contain the hydrocarbon sesquiterpene α-copaene, a powerful attractant to male medflies (Warthen & McInnis 1989; Flath et al. 1994), and α-copaene alone was found to increase mating success in male medflies (Shelly

2001). In light of this latter finding, in particular, it appears probable that α-copaene, alone or with other compounds, influenced male mating performance following exposure to the various plants and oils noted above.

The compound α-copaene occurs in a wide range of plant species, including corn (Köllner et al. 2004), wheat (Buttery et al. 1985), oaks (Vrkočová et al. 2000), and pine trees (Barnola et al. 1994) as well as many host plants of *C. capitata*, such as *Citrus* spp. (Nishida et al. 2000; Dou 2003). As the concentration of α-copaene and related mono- and sesquiterpenes vary among plant species, it is not known whether exposure to α-copaene-bearing plants (or their derived oils) will universally influence the mating behavior of male medflies. The goal of this study was to describe the results of mating trials conducted after male exposure to 2 previously untested sources of α-copaene, manuka oil from the New Zealand manuka tree, *Leptospermum scopariu* (Forst. & Forst.) and mango (*Mangifera indica* L.) fruits from cultivars in Hawaii and Guatemala. In addition, the relative attraction of males and females to manuka oil was compared in field-cage trials.

Materials and Methods

The methods employed are similar to those described previously (Shelly 2001; Shelly et al. 2004a). Consequently, an abbreviated description follows, and the aforementioned studies should be consulted for additional details. Also, the single experiment (involving mango exposure) conducted in Guatemala followed the same basic procedure used in Hawaii, consequently only methods unique to Guatemala are noted.

Hawaii: Study Insects

Because of low availability of wild flies, we used flies from recently established colonies (REC) derived from 500-1000 adults reared from field-collected fruits. For experiments involving manuka oil, the wild flies were derived from coffee berries (*Coffea arabica* L.) from the island of Kauai, and the flies tested were 3-4 generations removed from the wild. For experiments involving mango, the wild flies were derived from Jerusalem cherry (*Solanum capsicum* L.) from the island of Hawaii, and the flies tested were 5-6 generations removed from the wild. Adults of both colonies were provided with a sugar-protein mixture and water and a perforated vial for oviposition. Eggs were placed on standard larval diet (Tanaka et al. 1969), and pupae were sifted from vermiculite placed beneath the diet containers. Adults used in the mating trials were separated by sex within 24 h of eclosion and kept in screen-covered, plastic buckets (5-L volume, with cloth-sleeved-opening on side for transferring flies) with ample food and water.

Mass-reared, sterile males from a genetic sexing (temperature sensitive lethal, *tsl*) strain were obtained from the California Department of Food and Agricultural Fruit Fly Rearing Facility (Waimanalo, HI). The *tsl* males were dyed fluorescent pink and irradiated as pupae 2 d before eclosion and were maintained as adults in the same manner as the REC flies.

Manuka Oil—Source and Exposure Protocol for Mating Trials

Douglas et al. (2004) identified over 40 compounds in the essential oils of the New Zealand manuka, including many terpenoids. The oil used in the present study was obtained from Coast Biologicals Limited (Auckland, New Zealand) and, according to that company's specifications, contained 42-48 g/L of α-copaene. This concentration is approximately 1 order of magnitude greater than that recorded for α-copaene in ginger root oil (0.4%, Shelly 2001).

We conducted 5 experiments investigating the effect of manuka oil on male mating success during Apr-Jun 2006. REC flies were used exclusively in the first 2 experiments with treated REC males exposed to 100 µL (experiment 1) or 10 µL (experiment 2), competing against non-exposed (control) REC males for matings with REC females. In the remaining experiments, treated *tsl* males were exposed to manuka oil and competed against (non-exposed) REC males for matings with REC females. Doses of 100 and 10 µL were used to expose *tsl* males in experiments 3 and 4, respectively. In experiment 5, treated *tsl* males were also exposed to 100 µL but were prevented from physically contacting the oil-bearing source. For experiments 3-5, corresponding controls were run in which control, non-exposed *tsl* males competed against (non-exposed) REC males for REC females, thus allowing assessment of the impact of manuka oil exposure on the mating competitiveness of *tsl* males.

For exposure, we transferred 60 sexually mature males (REC males: 7-10 d old; *tsl* males: 5-8 d old) to a new bucket and, and using a microcapillary pipette, applied the oil to a small paper disc (5 mm diameter) resting in an aluminum-foil-lined Petri dish, which was then placed on the floor of the male-holding bucket. Exposure started at 0900 h and continued for 1 h. Aside from the final experiment, the oil-laden disk was uncovered during exposure. The behavior of males was not monitored systematically during exposure periods, but in frequent checks males were never seen touching the paper disk, an observation consistent with prior studies involving α-copaene or α-copaene-containing oils (Nishida et al. 2000; Shelly 2001). Rather, the oil acted as an arrestant, and males were generally quiescent. Nonetheless,

in the final experiment, we covered the Petri dish with nylon screening to guarantee that males did not contact the oil-laden paper disk. Upon removal of the oil, food and water were added to the bucket, and males were held for testing the following day. In all instances, the exposure procedure was conducted in an isolated room to prevent inadvertent exposure of other flies.

Mangos—Source and Exposure for Mating Trials

The mangos used for medfly exposure were obtained from trees of the 'Momi-K' and 'Pope' varieties at the University of Hawaii Agricultural Experiment Station (Waimanalo, HI; Warner 1972). Trace amounts of α-copaene were present in the peel of fruits (n = 5) sampled from these trees, but quantitative data on concentration is lacking (F. X. Webster, personal communication). Several thousand cultivars of mango exist (Nakasone & Paull 1998), and their volatile components vary considerably in composition and concentration (see references below). However, monoterpene and sesquiterpene hydrocarbons are the major volatile components of most mango cultivars, particularly among New World varieties, and may represent 70-90% of the total volatiles (Winterhalter 1991). A (non-exhaustive) survey of published studies revealed that, of 36 cultivars analyzed, 80% (29/36) contain α-copaene, usually in concentrations of 0.01-0.12 mg/kg (MacLeod & De Troconis 1982; Engel & Tressl 1983; MacLeod & Pieris 1984; MacLeod & Snyder 1985; Bartley & Schwede 1987; MacLeod et al. 1988; Idstein & Schreier 1985; Sakho et al. 1985; Cossé et al. 1995; Malundo et al. 1996, 1997; Ollé et al. 1998; Hernandez-Sanchez et al. 2001; Nair et al. 2003; Lalel et al. 2003; Zhu et al. 2003; Lebrun et al. 2004; Mahattanatawee et al. 2005; Pino et al. 2005; De Lourdes Cardenal et al. 2005). Even in those cases where α-copaene was not detected, a large number of related terpenes are invariably reported, and some of these (e.g., p-cymene and limonene) are known to be attractive to medfly males (Herández-Sánchez et al. 2001) or to elicit electroantennographic responses of medfly males (β-caryophyllene, Cossé et al. 1995). In sum, although quantitative chemical analyses are not available, we know that the mango fruits used for medfly exposure contained a rich mixture of terpenoid compounds, including α-copaene.

We ran 4 experiments in Hawaii examining the effect of mango fruit exposure on male mating success during Apr-May 2002. REC flies were used exclusively in the first 2 experiments, with treated, mango-exposed REC males competing against control, non-exposed REC males for matings with REC females either 1 d (experiment 6) or 3 d (experiment 7) after fruit exposure. In the remaining experiments, treated *tsl* males were exposed to ripe mango fruits and competed against (non-exposed) REC males for matings with REC females either 1 d (experiment 8) or 3 d (experiment 9) after fruit exposure. For experiments 8 and 9, corresponding controls were run in which control, apple-exposed *tsl* males competed against (non-exposed) REC males for REC females, thus allowing assessment of the impact of mango exposure on the mating competitiveness of *tsl* males.

For exposure, we transferred 60 sexually mature males to a screen cage (30 cm cube) and introduced 2 ripe mangos (fruits from the 2 cultivars used were not distinguished). Prior to use, we made 5 shallow cuts (2-4 cm long) into the skin of all fruits using a scalpel; α-copaene occurs in both the skin and pulp of mango fruits (Lalel et al. 2003). Exposure started at 0900 h and continued for 4 h. Upon removal of the fruits, food and water were added to the cage, and males were held until testing 1 or 3 d later. Control *tsl* males were handled in the same manner except that they were presented with 2 Granny Smith apples (*Malus sylvestris* Mill.) instead of mangos. Mango- and apple-exposure were performed in separate rooms to avoid aromatic 'contamination'.

Mating Trials

Mating trials were conducted at the USDA-ARS laboratory in Honolulu and followed the same protocol for experiments involving exposure to manuka oil or mangos. In all cases, we released 50 males of each competing type and 50 REC females (8-13 d old) at 0800 h in nylon-screen field cages containing 2 artificial trees and collected mating pairs over the next 4 h. In experiments involving REC males exclusively, treated and control individuals were marked 2-4 d before testing (in all cases, treated males were marked prior to oil or fruit exposure) with a dot of enamel paint on the thorax. In experiments involving *tsl* males, mated males were examined under a UV light and identified by the presence (*tsl*) or absence (REC) of pink dye. On a given test day, we ran 4 tents simultaneously. For experiments involving REC males exclusively, each cage contained treated and control REC males, and for experiments involving *tsl* males, 2 cages contained treated *tsl* males and 2 cages contained control *tsl* males competing against REC males. The particular cages used for treated and control *tsl* males were alternated between successive test days.

Comparisons of mating numbers were made with a t-test for pair wise comparisons and ANOVA for multi-sample tests as assumptions of normality (tested by using the Kolmogorov-Smirnov distribution with Lillefors correction) and homoscedasticity (tested by Levene's median test) were met in all cases (with only 2 exceptions in which case data were $\log_{10}$ transformed). Where comparisons involved proportions, values were arcsine transformed. Statistical tests were performed with SigmaStat (Version 2.0).

Manuka Oil—Attraction of Males and Females in Field Cage Trials

The attraction of medflies to manuka oil was examined by comparing the capture of males and females (from the coffee-derived REC described above) to manuka oil- versus water-baited Jackson traps suspended in the canopy of the artificial trees in the same field cages used in the mating trials. In each cage, we suspended 2 Jackson traps with sticky inserts. Each trap contained a paper disk near the center of the insert, and in 1 trap this disk contained 100 µL of manuka oil, and in the other the disk contained 100 µL of water. For a given field cage, the oil- and water-containing traps were placed at the same locations in all trials. On a given test day, 100 males or 100 females were released in a cage (2 cages per sex per day) at 0900 h, and traps were collected and scored 3 h later. The sex released in a given cage was alternated between successive test days. Tests were run in Jun-Jul 2006; a total of 8 replicates were run per sex. Pair wise comparisons were made with the *t*-test (raw data) as parametric assumptions were met.

Guatemala

A single experiment assessing the effect of mango exposure on male mating success was conducted during Mar 2003 in Guatemala. Wild flies reared from field-collected coffee were used exclusively, and individuals of both sexes were 9 d old when tested. Treated males were placed in screen cages along with 2 ripe mangos (variety unknown) for 3 h (0900-1200 h) on the day preceding the mating trial; control males were not exposed to fruit of any type. Treated and control males were marked with paint as described above 2 d before testing. Mating trials were conducted in field cages erected over individual coffee plants at Finca San Augustin, near Guatemala City. In each field cage, we released 100 treated males, 100 control males, and 100 females at 0700 h and collected mating pairs over the next 5 h.

Results

Hawaii: Mating Trials

In tests involving REC males exclusively (experiments 1 and 2), exposure to manuka oil increased mating success for both doses tested (Table 1A). In fact, the mating frequencies of treated REC males were nearly identical for the 2 doses, both in terms of the number ($t = 0.2, P > 0.05$) and the proportion ($t = 0.4, P > 0.05$) of matings obtained.

Similarly, in experiments involving REC and *tsl* males (experiments 3-5), exposure to manuka oil enhanced the mating success of the *tsl* males (Table 1B). In all 3 experiments, REC males achieved significantly more matings than treated (oil-exposed) *tsl* males. However, in all 3 cases, the treated *tsl* males obtained a significantly greater number of matings than the control (non-exposed) *tsl* males (experiment 3: $t = 7.0, P < 0.001$; experiment 4: $t = 3.1, P < 0.01$; experiment 5: $t = 7.1, P < 0.001$). Correspondingly, in all 3 cases, the treated *tsl* males accounted for a significantly greater proportion of the total matings than the control (non-exposed) *tsl* males (experiment 3: $t = 6.9$, $P < 0.001$; experiment 4: $t = 3.2, P < 0.01$; experiment 5: $t = 4.4$, $P < 0.001$). Among treated *tsl* males, there was no detectable effect of dose or oil-accessibility (covered or not) on mating success; there was no significant variation among treated *tsl* males in experiments 3-5 in either the numbers ($F_{2, 21} = 2.3, P > 0.05$, ANOVA) or proportions ($F_{2, 21} = 0.85, P > 0.05$, ANOVA) of matings obtained.

In contrast to the manuka oil, exposure to mangos had no apparent effect on the mating success of REC or *tsl* males. In the experiments involving REC males exclusively (experiments 6 and 7), there was no difference in the numbers of matings obtained by mango-exposed and non-exposed males either 1 or 3 d after treatment, and fruit-exposed and non-exposed males accounted for approximately 50% of the total matings. In the experiments involving REC and *tsl* males (experiments 8 and 9), REC males obtained significantly more matings than mango-exposed *tsl* males, and there was no significant difference between mango- and apple-exposed *tsl* males in either the number (experiment 8: $t = 0.4$; experiment 9: $t = 0.9$) or proportion (experiment 8: $t = 0.5$, experiment 9: $t = 0.4, P > 0.05$ in all cases).

Attraction to Manuka Oil

Significantly greater numbers of males than females were captured in traps baited with manuka oil (41.2 ± 3.1 versus 5.5 ± 2.1, respectively, (mean ± SE), $t = 9.0, P < 0.001$) or water (12.0 ± 2.2 versus 3.3 ± 0.9, respectively, $P < 4.2, P < 0.001$). Significantly more males were captured in traps baited with manuka oil than water ($t = 7.3$, $P < 0.001$), while for females there was no significant difference between trap types ($t = 1.6, P > 0.05$).

Guatemala

Exposure to mangos had no effect on male mating success in the single experiment run in Guatemala. On average, mango-exposed males obtained 31.0 ± 2.1 matings (mean ± SE) compared to 32.5 ± 1.4 for control, non-exposed males ($t = 0.6, P > 0.05, n = 6$).

Discussion

Consistent with tests involving ginger root oil (Shelly 2001; Shelly et al. 2002, 2003, 2004b) and orange oil (Shelly et al. 2004a, 2007), exposure to manuka oil enhanced mating success in experi-

TABLE 1. RESULTS OF MATING TRIALS CONDUCTED IN HAWAII TESTING THE INFLUENCE OF MANUKA OIL WITH (A) REC MALES EXCLUSIVELY OR (B) REC AND *tsl* MALES. VALUES ARE MEANS ± SE. FOR A GIVEN EXPERIMENT, *t* VALUES REFER TO COMPARISONS MADE BETWEEN (A) TREATED AND CONTROL REC MALES OR (B) REC MALES AND TREATED OR CONTROL *tsl* MALES. TWELVE REPLICATES WERE CONDUCTED FOR EXPERIMENTS 1 AND 2, AND 8 REPLICATES WERE CONDUCTED FOR EXPERIMENTS 3-5.

A. REC males

Experiment	Male type	Matings per replicate	t[1]	% Matings treated males
1	Treated, 100 µl oil	20.1 (1.5)		
	Control, non-exposed	13.7 (1.9)	2.6*	60.4
2	Treated, 10 µl oil	20.5 (1.2)		
	Control, non-exposed	14.2 (0.8)	4.6***	59.0

B. REC-*tsl* males

Experiment	Male type	Matings per replicate	t	% Matings *tsl* males
3	REC, non-exposed	20.7 (1.7)		
	Treated *tsl*, 100 µl oil	14.5 (0.9)	3.2**	41.2
	REC, non-exposed	21.7 (1.8)		
	Control *tsl*, non-exposed	4.7 (1.7)	6.4***	17.7
4	REC, non-exposed	18.9 (2.7)		
	Treated *tsl*, 10 µl oil	10.3 (2.1)	2.5*	35.3
	REC, non-exposed	24.9 (2.6)		
	Control *tsl*, non-exposed	3.0 (0.9)	7.8***	10.7
5	REC, non-exposed	19.3 (2.5)		
	Treated *tsl*, 100 µl oil[covered]	11.1 (1.3)	3.5**	36.5
	REC, non-exposed	24.0 (2.7)		
	Control *tsl*, non-exposed	3.6 (0.5)	7.4***	13.0

[1]Significance levels: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

ments with wild-like or *tsl* males. Increased mating success was observed even when the exposed males were prevented from contacting the manuka oil, revealing that (as also noted for the other oils tested) aroma alone was sufficient to boost mating performance. Despite a much higher concentration of α-copaene (~10-fold greater), manuka oil enhanced the mating frequency of *tsl* males to about the same level as ginger root oil. In the present study, manuka oil increased mating frequency from 11-18% for control *tsl* males to 35-41% for treated *tsl* males, whereas the corresponding increases (from control *tsl* to treated *tsl* proportion of total matings in competition with wild males) for tests involving ginger root oil were 16% to 30% (Shelly et al. 2002), 16% to 39% (Shelly et al. 2002), and 19% to 40% (Shelly et al. 2003) and for a test involving orange oil was 26% to 43% (Shelly et al. 2007). The greatest increase recorded thus far, in fact, has been noted for ginger root oil, where the relative mating success increased from 23-27% for control *tsl* males to 43% - 56% for treated *tsl* males in a series of tests involving wild Hawaiian flies (Shelly et al. 2004b). The finding that males were more attracted to manuka oil than females was also recorded for ginger root oil (Shelly & Pahio 2002).

Although the underlying mechanism remains unknown, studies conducted in a large field enclosure (Shelly 2001) and in a laboratory wind-tunnel (Papadopoulos et al. 2006) suggest that exposure to ginger root or orange oil (and presumably manuka oil as well) does not affect the attractiveness of the male sex pheromone to females. Instead, preliminary tests suggest that the aroma of ginger root oil interacts with the male exoskeleton in some way to produce a scent attractive to females. Females whose antennae were surgically removed do not discriminate between ginger root oil-exposed males and non-exposed males. However, males whose antennae were removed prior to exposure to ginger root oil have a mating advantage over intact males not given access to the oil. These results suggest that female preference is based on olfactory cues associated with exposed males but that male emission of these preferred cues does not depend on male ability to smell (and internally process) the oil aroma. Whether tests involving orange or manuka oil would yield the same results remains unknown.

In contrast to the manuka oil, exposure to ripe mangos did not boost male mating success in tests conducted in Hawaii or Guatemala. The absence of mating enhancement may have reflected very

TABLE 2. RESULTS OF MATING TRIALS CONDUCTED IN HAWAII TESTING THE INFLUENCE OF MANGOS WITH (A) REC MALES EXCLUSIVELY OR (B) REC AND *tsl* MALES. VALUES ARE MEANS ± SE. FOR A GIVEN EXPERIMENT. VALUES OF *T* REFER TO COMPARISONS MADE BETWEEN (A) TREATED AND CONTROL REC MALES OR (B) REC MALES AND TREATED OR CONTROL *tsl* MALES. TEN REPLICATES WERE CONDUCTED FOR EXPERIMENTS 6-9.

A. REC males

Experiment	Male type	Matings per replicate	t[1]	% Matings treated males
6	Treated, mangos, 1 d pre-test	12.7 (1.1)		
	Control, non-exposed	12.3 (0.8)	0.3^{NS}	50.8
7	Treated, mangos, 3 d pre-test	11.4 (0.8)		
	Control, non-exposed	12.2 (0.9)	0.1^{NS}	48.3

B. REC-*tsl* males

Experiment	Male type	Matings per replicate	t	% Matings *tsl* males
8	REC, non-exposed	21.6 (2.4)		
	Treated *tsl*, mangos, 1 d pre-test	7.3 (0.9)	6.2***	25.3
	REC, non-exposed	22.5 (1.6)		
	Control *tsl*, apples, 1 d pre-test	6.9 (1.1)	6.6***	23.5
9	REC, non-exposed	19.9 (2.2)		
	Treated *tsl*, mangos, 3 d pre-test	4.4 (0.4)	7.2***	18.1
	REC, non-exposed	24.7 (2.5)		
	Control *tsl*, apples, 3 d pre-test	6.5 (0.6)	6.9***	20.8

[1]Significance levels: NS—not significant ($P > 0.05$), ***$P < 0.001$.

small amounts of α-copaene in the mango cultivars tested or a particular mixture of compounds that diminished or blocked α-copaene's effect on the male medflies, or absence of another compound(s) that acts synergistically with α-copaene to promote male mating ability. Consequently, we tentatively conclude that exposure to α-copaene-containing fruits does not 'automatically' result in increased mating success of male medflies.

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