Exposure to α-Copaene–Containing Fruits Enhances the Mating Success of Males from a Mass-Reared, Genetic Sexing Strain of the Mediterranean Fruit Fly (Diptera: Tephritidae)

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Abstract. Recent research has demonstrated that exposure to ginger root oil (*Zingiber officinale* Roscoe, which contains α-copaene) significantly increases the mating success of mass-reared, sterile males of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). The present study had two main objectives. First, I assessed whether mass-reared males gain an advantage through exposure to natural sources of α-copaene following their release into the environment. Sterile males from a genetic sexing (temperature sensitive lethal, *tsl*) strain were exposed to fruits of guava trees (*Psidium guajava* L.) and the fruits and leaves of orange trees (*Citrus sinensis* L.) and then competed with wild-like males for copulations with wild-like females. Exposure to oranges increased the mating success of *tsl* males in tests conducted 1 or 3 d after exposure, but exposure to orange tree leaves had no effect. Exposure to guavas yielded inconsistent results: *tsl* males tested 3 d after exposure showed enhanced mating success, but those tested 1 d after exposure did not. Second, to more closely mimic conditions characteristic of sterile male releases, I measured the mating success of mass-reared males (exposed or unexposed to a source of α-copaene) when competing against wild males exposed to a natural source of α-copaene. In these tests, exposed *tsl* males were outcompeted by wild-like males but to a lesser degree than non-exposed, *tsl* males. Implications of these findings for the sterile insect technique against *C. capitata* are discussed.

Key Words: *Ceratitis capitata,* male mating success, plant-insect interaction, sterile insect technique

Introduction

Plant derived chemicals often play an important role in the mating behavior of male phytophagous insects. In some species, males ingest specific compounds as larvae (Conner et al. 1981, Lofstedt et al. 1989) or adults (Pliske 1975, Krasnoff and Dussourd 1989) and utilize them to synthesize the sex pheromone. For example, adult males of a bark beetle, *Dendroctonus ponderosae* Hopkins, use a-pinene from host trees in the synthesis of the pheromone trans-verbanol (Hughes 1973). Plant volatiles may also serve to stimulate the release of male sex pheromone (Jaffe et al. 1993). Alternatively, males may incorporate plant compounds in nuptial gifts that are transferred to females during mating. In an elegant example, males of the moth *Utetheisa ornatrix* L. sequester pyrrolizidine alkaloids (PA) as larvae from food plants and transfer PA to females during mating, who, in turn, transfer PA to the eggs (Eisner and Meinwald 1995). The PA confer protection against predators to the female (Gonzalez et al. 1999) as well as the eggs and larvae (Eisner and Eisner 1991, Hare and Eisner 1993).

Adult ingestion of specific plant compounds appears to influence male mating behavior in several species of tephritid fruit flies. In the oriental fruit fly, *Bactrocera dorsalis* (Hendel), males that feed on flowers containing methyl eugenol produce a more attractive pheromone (Shelly 2001a) and obtain more matings than males deprived of such flowers (Shelly 2002). Nishida et al. (1988, 1997) revealed that metabolites of methyl eugenol are sequestered in the male rectal gland, the site of pheromone synthesis. Plant derived chemicals appear to have a similar function in the melon fly, *B. cucurbitae* (Coquillett) (Shelly 2000, Nishida et al. 1993). Likewise, males of the Mediterranean fruit fly (or medfly), *Ceratitis capitata* (Wiedemann), exposed to the branches and ripe fruits of guava (*Psidium guajava* L.; Shelly and Villalobos 2004) or the leaves and fruits of orange (*Citrus sinensis* L.; Papadopoulos et al. 2001, Shelly et al. 2004) have a mating advantage over males denied access to these plants. It is not known what chemical mediates male medfly behavior, but several lines of evidence suggest that the hydrocarbon sesquiterpene α-copaene is involved: i) the compound is highly attractive to wild male medflies (Flath 1994a,b), ii) pure samples of the compound confer a mating advantage over non-exposed males (Shelly 2001b), and iii) the plants known to confer a male mating advantage all contain this chemical (Nishida et al. 2000 and references therein).

Additional research on the medfly has explored the possibility that pre-release exposure to essential oils containing α-copaene might increase the mating competitiveness of mass-reared males in sterile release programs (Shelly and McInnis 2001). This work has demonstrated that, at least in field cage tests, exposure to the odor of commercially available, ginger root oil (*Zingiber officinale* Roscoe) significantly increases the mating success of sterile males from a widely used, genetic sexing strain (see below) in competition with wild males for wild females. In Hawaii, for example, exposure to ginger root oil reversed the outcome of mating competition: sterile males obtained only 26% of all matings in the absence of chemical exposure compared to 75% of the total matings after exposure to ginger root oil (Shelly and McInnis 2001).

The present study had two main objectives. As all previous research with natural sources of α-copaene has involved wild flies (or flies from "young" laboratory colonies; Papadopoulos et al. 2001, Shelly and Villalobos 2004, Shelly et al. 2004), the first objective of this study was to evaluate whether exposure to natural sources of α-copaene might also confer a mating advantage to mass-reared males following their release into the environment. While generating useful data, these tests did not mimic closely the conditions characteristic of sterile male releases, because the wild males (against which the mass-reared males competed) were not given access to any natural sources of α-copaene. Thus, the second objective of the study was to monitor the mating success of mass-reared males (exposed or unexposed to a source of α-copaene) when competing against wild males exposed to a natural source of α-copaene. Implications of these findings for sterile release programs are discussed.

Materials and Methods

Study insects. Because wild flies were not available in sufficiently large numbers, "wild-like" flies were used in this study. These flies were from a laboratory colony started with 200-300 adults reared from fruits of Jerusalem cherry, *Solanum capsicum* L., collected in Hawaii Volcanoes National Park. Adults were held in screen cages and provided a sugar-protein (yeast hydrolysate) mixture (3:1 v:v), water, and an oviposition substrate (perforated plastic vials containing small sponges soaked in lemon juice). Eggs were placed on standard larval medium (Tanaka et al. 1969) in plastic containers placed over vermiculite for pupation. Adults used in the experiments were separated by sex within 24 h of eclosion

(well before reaching sexual maturity at 5–7 d of age; Shelly, unpublished data), and kept in screen-covered buckets (5 liter volume; 100–150 flies per bucket) with ample food (sugar-protein mixture) and water. When used in the mating tests, wild-like flies were 7–13 d old. The flies were maintained at 20–24˚C and 60–90% RH and received both natural and artificial light under a photoperiod of approximately 12:12 (L:D) When used in the present study, wild-like flies were 6–9 generations removed from the wild.

Mass-reared flies were from a temperature sensitive lethal (*tsl*) strain (Vienna-7/Tol-99) produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility, Waimanalo, Oahu. Like other *tsl* strains, Vienna-7/Tol-99 possesses a sex-linked mutation, such that treating eggs with high temperature kills female zygotes, thereby allowing production of males exclusively (Franz et al. 1996). Larvae of the *tsl* mass-reared strain were reared on the standard diet (Tanaka et al. 1969), and males used in the present study were irradiated as pupae 2 d before eclosion in air at 150 Gy of gamma irradiation from a ^{137}Cs source. Irradiated pupae were placed in 5-liter plastic buckets (300–400 pupae per bucket), and the adults were maintained in the manner described above for the wild-like flies. Males of this strain attain sexual maturity at 3–4 d of age (K. Fisher, personal communication). When used in the mating tests, *tsl* males were 6–10 d old.

Exposure of males to fruits, leaves, or ginger root oil. Over the entire study, I performed six experiments in which *tsl* males competed against wild-like males for wild-like females. These experiments were of three basic types. In the first type, wild-like and *tsl* males were tested without exposure to any natural source of α-copaene (experiment 1). In the second type (experiments 2–4), *tsl* males were exposed to plants containing α-copaene (treated males) or plants lacking α-copaene (control males), whereas wild-like males were not exposed to any plants at all. Treated *tsl* males were exposed to oranges (experiment 2), guavas (experiment 3), or orange tree leaves (experiment 4), whereas control *tsl* males were exposed to apples (*Malus sylvestris* Mill.; experiments 2–3) or macadamia tree leaves (*Macadamia integrifolia* Maiden and Betche; experiment 4). Exposure to guava tree leaves had no effect on the mating success of wild-like males (Shelly and Villalobos 2004) and thus were not tested in the present study. In the third type, both treated *tsl* and wild-like males were exposed to a source of α-copaene before testing. In experiment 5, both treated *tsl* and wild-like males were exposed to oranges (control *tsl* males were exposed to apples as before). In experiment 6, treated *tsl* males were exposed to ginger root oil and wild-like males were again exposed to oranges (control *tsl* males were not exposed to any source of α-copaene).

For fruit exposure, 60 *tsl* (5–7 d old) or 60 wild-like (7–10 d old) males were placed in screen cages (30 cm cubes) between 0900–1000 hrs with two Valencia oranges or two guava fruits, collected 3 h later, and kept in the laboratory (with food and water) until tested 1 or 3 d later. Males exposed to α-copaene-containing fruits were referred to as treated males. Control *tsl* males (5–8 d old) were handled in the same manner except that they were presented with two Granny Smith apples, which are not known to contain α-copaene. Oranges and apples were purchased in a supermarket and then rinsed in water and dried prior to use. Ripe guava fruits were collected at the field site (see below) and used immediately upon return to the laboratory. Before exposure, I made five shallow cuts (2–4 cm long) into the peel of all fruits using a scalpel. Treated and control males were exposed to fruits in separate rooms.

For leaf exposure, 60 *tsl* males (5–7 d old) were placed in screen cages (30 cm cubes) between 0900–1000 hrs with 20–30 orange tree leaves, collected 3 h later, and kept in the laboratory (with food and water) until tested 1 or 3 d later. Leaves were placed on a chicken-wire screen raised above the cage floor, allowing males access to the top and bottom leaf surfaces. Males exposed to these α-copaene-containing leaves were referred to as treated males. Control *tsl* males (5–7 d old) were handled in the same manner except that they were

presented with 10–15 macadamia tree leaves, which are not known to contain α-copaene. All leaves were collected at the field site (see below) and used immediately upon return to the laboratory. Treated and control males were exposed to leaves in separate rooms.

Exposure to ginger root oil was accomplished using eclosion (so-called PARC) boxes typical of area-wide control programs (e.g., California, Guatemala). Approximately 40,000 irradiated pupae were placed in these boxes 2 d prior to peak emergence, and emerged adults were fed sugar agar gel. To obtain treated males, I applied 1 ml of the oil to blotter paper, which was then placed on the screened opening in the lid of the eclosion box. Ginger root oil was applied when the adults were 3 d old and left in place for 24 h. No oil was applied to eclosion boxes holding control males, and these boxes were held in a separate room to avoid inadvertent exposure to the oil's aroma. Treated males (and their associated control males) were tested 1 or 3 d after the ginger root oil was removed.

Mating trials. Mating tests were conducted at the Agricultural Experiment Station of the University of Hawaii, Waimanalo, or the USDA-ARS-PBARC laboratory, Honolulu. One day prior to testing, the wild-like males were marked by cooling them for several minutes and then placing a dot of enamel paint on the mesonotum. This procedure has no obvious adverse effects, and males resumed normal activities within minutes of handling. In all experiments, I released 100 wild-like males, 100 wild-like females, and 100 treated or 100 control *tsl* males between 0730–0830 hrs (males were released 15 min before females) in nylon-mesh field-cages (3 m diameter, 2.5 m high) that contained two, artificial trees (each 2 m tall with approximately 450 leaves resembling those of *Ficus benjamina* L.). Artificial trees were used, because they provided a chemically neutral substrate on which the flies displayed the entire complement of natural activities. The cages were monitored for 3 h, mating pairs were collected in vials, and the males identified. Individuals of both sexes were virgins when tested, and new flies were used in every trial.

Statistical analyses. For a given experiment, the numbers of matings obtained by wild-like and *tsl* males were compared using the *t*-test as the assumptions of normality and equal variances were met in all instances. Multi-group comparisons were made using 1-way ANOVA (proportions were arcsine transformed values) as the underlying parametric assumptions were again met (excepting comparisons involving the relative mating success of treated *tsl* males in experiments 2–4, where the Kruskal-Wallis test was used).

Results

In the absence of any plant exposure (experiment 1), wild-like males outcompeted *tsl* males and, on average, obtained nearly three times as many matings per replicate as *tsl* males (Table 1). Following exposure to oranges (experiment 2), *tsl* males had similar mating success as wild-like males in trials conducted 1 or 3 d after exposure. In contrast, the mating success of control *tsl* males (exposed to apples) was significantly lower than that observed for wild-like males in both experiments 2a and 2b. The results obtained with guavas were less clear-cut. In tests conducted 3 d after exposure (experiment 3b), *tsl* males mated with equal frequency as wild-like males. However, in tests conducted 1 d following exposure (experiment 3a), *tsl* males were outcompeted by wild-like males and achieved, on average, only about half as many matings as wild-like males. Wild-like males had a significant mating advantage over control *tsl* males in both the 1- and 3-d post exposure trials. The number of matings per replicate did not vary significantly among the apple-exposed males in experiments 2 and 3 and the (unexposed) *tsl* males in experiment 1 ($F = 0.40$, $df = 4, 50$, $P > 0.05$), indicating that exposure to apples represented a valid control.

In contrast to the fruits, exposure to orange tree leaves had no apparent effect on the mating success of *tsl* males (experiment 4; Table 1). Wild-like males obtained significantly

Table 1. Number of matings obtained by wild-like and *tsl* males in field-cage tests[a].

Experiment,	*tsl* exposure	Post-exposure interval (d)	Matings per replicate Wild-like	*tsl*	t
1	None	Not applicable	31.6 (10.4)	11.6 (4.6)	5.9***
2a	Oranges (T)	1	15.8 (7.1)	15.5 (7.0)	0.09^{NS}
	Apples (C)		25.1 (7.7)	12.3 (5.2)	5.3***
2b	Oranges (T)	3	24.4 (7.7)	18.6 (7.8)	1.8^{NS}
	Apples (C)		35.5 (10.8)	16.3 (6.0)	6.5***
3a	Guavas (T)	1	26.3 (14.3)	12.7 (3.3)	3.1**
	Apples (C)		35.9 (9.0)	12.4 (5.2)	7.1***
3b	Guavas (T)	3	23.2 (10.4)	22.9 (8.2)	0.1^{NS}
	Apples (C)		30.3 (5.5)	13.8 (7.1)	5.3***
4a	Orange leaves (T)	1	33.8 (10.2)	19.0 (4.8)	3.4**
	Macad. leaves (C)		26.1 (8.4)	12.7 (9.3)	4.3***
4b	Orange leaves (T)	3	31.3 (5.6)	13.9 (4.6)	7.6***
	Macad. leaves (C)		29.0 (9.1)	17.7 (4.9)	4.3***

[a]Wild-like males were not exposed to any plants in experiments 1–4, and *tsl* males were not exposed to any plants in experiment 1. In experiments 2 and 3, treated (T) *tsl* males were exposed to α-copaene-containing orange or guava fruits, respectively, and control (C) *tsl* males were exposed to apples. In experiment 4, treated (T) *tsl* males were exposed to α-copaene-containing orange leaves, and control (C) *tsl* males were exposed to macadamia leaves. Eleven replicates were conducted for experiments 1–3 ($df = 20$ in accompanying *t*-tests), and 7 replicates were conducted for experiment 4 ($df = 12$ in accompanying *t*-tests). Means ± 1 SD are given. Significance levels: *P < 0.05; **P < 0.01; ***P < 0.001; ^{NS}P > 0.05.

more matings per replicate than treated *tsl* males in tests conducted 1 or 3 d following exposure, and there was no significant difference in the number of matings achieved by treated and control *tsl* males for either post-exposure interval (1 d - $t = 0.3$, $P > 0.05$; 3 d – $t = 0.4$, $P > 0.05$). In addition, there was no difference in the number of matings obtained per replicate for control *tsl* males exposed to macadamia leaves in experiment 4 (a and b) and the (unexposed) *tsl* males in experiment 1 ($F = 0.61$, $df = 2, 22$, $P > 0.05$), indicating that exposure to macadamia leaves represented a valid control.

Variation in the mating competitiveness of *tsl* males over these four experiments is perhaps best illustrated by plotting the relative mating success of treated and control *tsl* males (Fig. 1). On average, control *tsl* males accounted for 25–33% of total matings per replicate over experiments 1–4, and there was no significant variation in relative mating success of control *tsl* males among these experiments ($F = 0.76$, $df = 2, 62$, $P > 0.05$). Likewise, when only treated *tsl* males exposed to fruits were considered (experiments 2 and 3), relative mating success did not vary significantly among experiments (36–50%; $H = 7.3$, $df = 3$, $P >$

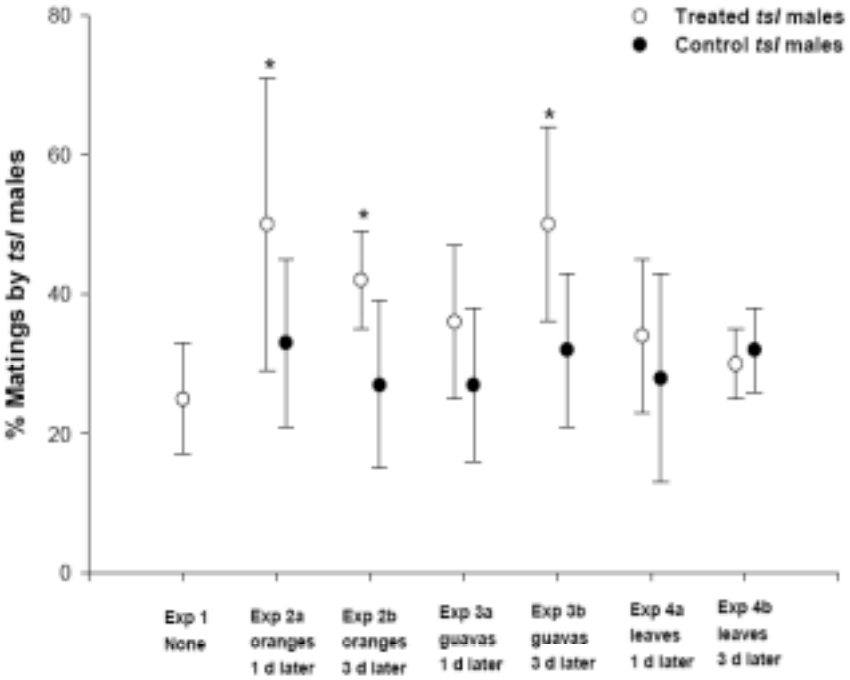

Figure 1. Proportion of matings obtained by treated and control *tsl* males in experiments 1–4. Plant structure to which treated *tsl* males were exposed ('leaves' in experiment 4 refers to orange tree leaves), and interval between exposure and testing are given along the abscissa. Control males were not exposed to plant structures (experiment 1) or were exposed to plant structures presumably lacking α-copaene (experiments 2–4). See text for details. Means ± 1 SD are given. Asterisks denote instances where relative mating success differed significantly between treated and control males (t-test, arsine transformed values, $P < 0.05$).

0.05). However, when the analysis also included treated *tsl* males exposed orange leaves, the relative mating success of treated *tsl* males was found to vary significantly among experiments ($H = 16.1$, $df = 5$, $P < 0.01$, with Dunn's multiple comparison test identifying a single significant difference between experiment 3b (treated *tsl* males tested 3 d after guava exposure) and 4b (treated *tsl* males tested 3 d after orange leaf exposure; $P < 0.05$).

Experiments 5 and 6 were designed to reflect the more realistic scenario in which wild-like males were also exposed to a source of α-copaene (oranges, in this case) prior to mating competition. Following exposure to oranges (experiment 5), wild-like males obtained significantly more matings than treated *tsl* males (Table 2). This result differs from that obtained in experiment 2a, where treated *tsl* males alone were exposed to oranges and had equivalent mating success as wild-like males. Thus, *tsl* males were at a competitive disadvantage when neither (experiment 1) or both (experiment 5) male types were exposed to oranges but had similar mating success to wild-like males when they alone were exposed to oranges (experiment 2). Although treated *tsl* males were outcompeted in experiment 5,

Table 2. Number of matings obtained by wild-like and *tsl* males in field-cage tests[a].

Expt.	Male exposure		Post-exposure interval (d)	Matings per replicate		
	tsl	Wild-like		Wild-like	*tsl*	*t*
5	Oranges (T)	Oranges	1	38.0 (4.9)	23.1 (5.9)	6.1***
	Apples (C)	Oranges		41.2 (7.4)	14.2 (5.5)	9.2***
6a	GRO (T)	Oranges	1	34.0 (7.1)	21.3 (7.6)	3.9**
	None (C)	Oranges		38.6 (4.2)	12.9 (8.2)	8.8***
6b	GRO (T)	Oranges	3[b]	29.5 (6.7)	24.1 (3.5)	2.2*
	None (C)	Oranges		35.3 (6.9)	16.8 (8.6)	5.3***

[a]In Experiment 5, both treated *tsl* and wild-like males were exposed to α-copaene-containing orange fruits, and control (C) *tsl* males were exposed to apples. Ten replicates were conducted for Experiments 5 and 6 ($df = 18$ in accompanying *t*-tests). Means ± 1 SD are given. Significance levels: *P < 0.05; **P < 0.01; ***P < 0.001; ^{NS}P > 0.05.
[b]Refers to *tsl* males; wild-like males were tested 1 d after exposure to oranges as in experiments 5 and 6a.

exposure to oranges nonetheless lessened the mating advantage of wild-like males. In experiment 5, treated *tsl* males obtained significantly more matings ($t = 3.5$, $df = 18$, $P < 0.01$) and accounted for a significantly greater proportion of the total matings (38% vs. 26%, $t = 3.3$, $df = 18$, $P < 0.01$) than control males. The mating success of control *tsl* males in experiment 5 (exposed to apples) did not differ significantly from that of non-exposed *tsl* males in experiment 1 in absolute (number of matings obtained; $t = 1.4$) or relative (proportion of matings obtained; $t = 0.2$) terms ($df = 19$ and $P > 0.05$ in both cases).

Results of experiment 6 were completely analogous to those obtained in experiment 5 (Table 2). That is, following exposure to oranges, wild-like males achieved significantly more matings than *tsl* males exposed to ginger root oil either 1 or 3 d prior to testing. In an earlier study (Shelly et al. 2004), *tsl* males exposed to ginger root oil in the manner used here displayed similar mating success to wild-like males not exposed to any source of α-copaene. Thus, similar to outcome of experiment 5, *tsl* males were at a competitive disadvantage when neither (experiment 1) or both (experiment 6) male types were exposed to a source of α-copaene but had similar mating success to wild-like males when they alone were exposed to a source of α-copaene (i.e., ginger root oil; Shelly et al. 2004). Likewise, treated *tsl* males were outcompeted in experiment 6, but exposure to ginger root oil lessened the mating advantage of wild-like males. In experiment 6, treated *tsl* males obtained significantly more matings ($t = 2.4$ and 2.7 for experiments 6a and 6b, respectively, in both cases, df = 18, $P < 0.05$) and accounted for a significantly greater proportion of the total matings (experiment 6a: 38 vs. 23%, $t = 2.7$, $P < 0.05$; experiment 6b: 45% vs. 30%, $t = 3.1$, $P < 0.01$; $df = 18$ in both tests) than control males. The mating success of control *tsl* males in experiment 6a and 6b (not exposed to ginger root oil) did not differ significantly from that observed for non-exposed *tsl* males in experiment 1 in absolute ($F = 1.5$, $df = 2, 28$, $P > 0.05$) or relative ($F = 0.9$, $df = 2, 28$, $P > 0.05$) terms.

Discussion

In the Mediterranean fruit fly, sterile males from a mass-reared, genetic sexing strain generally derived a benefit from exposure to natural sources of α-copaene when competing against non-exposed, wild-like males for copulations with wild-like females (Table 1). As found previously for wild-like males (Shelly et al. 2004), exposure to oranges boosted the mating success of *tsl* males in mating trials conducted 1 or 3 d following exposure. Similarly, like wild-like males (Shelly and Villalobos 2004), exposure to guavas enhanced the mating performance of *tsl* males, although a significant increase was noted only 3 d (and not 1 d) following exposure. Unlike wild-like males (Shelly et al. 2004), however, exposure to orange tree leaves had no significant effect on the mating success of *tsl* males.

Exposure to oranges or ginger root oil also enhanced the performance of *tsl* males when competing against orange fruit-exposed, wild-like males (Table 2). In these tests, however, wild-like males had a mating advantage even over the exposed *tsl* males. This finding reveals that exposure to α-copaene, while influential, is not the sole determinant of male mating success and that other factors (e.g., genetically mediated differences in male sexual advertisement) are involved. Nonetheless, exposure to an α-copaene source did improve the performance of *tsl* males as treated males obtained significantly more matings than control males. Thus, even where wild males have access to natural sources of α-copaene, pre-release exposure to ginger root oil would presumably still increase the effectiveness of sterile male releases.

The mechanism underlying elevated mating success following exposure to a source of α-copaene remains unknown. Exposure to ginger root oil results in increased pheromone calling by male medflies. However, the relative increase in mating frequency far exceeds that observed for signaling, indicating that other factors are also involved (Shelly 2001b). Evidence from field (Shelly 2001b) and wind tunnel (Papadopoulous et al., unpublished data) experiments reveals that females are attracted equally to the sex pheromone of ginger root oil-exposed and non-exposed males, indicating that components of the oil's aroma are not used as precursors in pheromone synthesis. Analysis of videotaped courtship bouts (Briceno and Shelly, unpublished data) suggests that wild females more rapidly "accept" or "cooperate" with sterile males exposed to ginger root oil. The durations of pre-mounting activities, such as wing vibration and buzzing and head rocking, were generally lower for ginger root oil-exposed males than control males. Whether this finding reflects differences between treated and control males in the rate and/or form of certain courtship displays or in close-range olfactory cues (e.g., "perfumed" versus "normal" male exoskeleton) remains unknown.

Regardless of the mechanism, the present findings indicate that natural sources of α-copaene may improve the effectiveness of sterile males of *C. capitata*, thus compensating, to some degree, for the low mating competitiveness inherent in mass-reared strains (e.g., Lance et al. 2000). This suggests, in turn, that the availability of α-copaene in a release area might affect the mating competitiveness of sterile males, e.g., sterile males from the same rearing facility may (all else being equal) obtain more matings with wild females in orange groves (α-copaene-rich areas) than in coffee fields (presumably α-copaene-poor areas). This notion can be tested experimentally by placing oranges or coffee berries in the canopy of artificial trees in separate, large field enclosures, releasing the same numbers of sterile males and wild flies in the two enclosures, and then comparing the level of egg sterility achieved in the two treatments. Although identifying such environmental influences on the effectiveness of the sterile insect technique is useful, the possibility of natural enhancement of sterile male mating success (through exposure to specific plants) in no way negates the potential usefulness of pre-release exposure to ginger root oil in the sterile insect technique, because this procedure eliminates the "need" for sterile males to locate chemical sources in the environment (thereby eliminating time and energy costs associated with searching) and guarantees that sterile males benefit fully from exposure to a performance-enhancing essential oil.

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Literature Cited

Conner, W. F., T. Eisner, R. K. Vandermeer, A. Guerrero and **J. Meinwald.** 1981. Precopulatory sexual interaction in an arctiid moth (*Utetheisa ornathrix*): role of a pheromone derived from dietary alkaloids. Behav. Ecol. Sociobiol. 9: 227–235.

Eisner, T. and **M. Eisner.** 1991. Unpalatability of the pyrrolizidine alkaloid-Containing moth *Utetheisa ornathrix*, and its larva, to wolf spiders. Psyche 98: 111–118.

Eisner, T. and **J. Meinwald.** 1995. The chemistry of sexual selection. Proc. Natl. Acad. Sci. (USA) 92: 50–55.

Flath, R. A., R. T. Cunningham, T. R. Mon, and **J. O. John.** 1994a. Male lures for Mediterranean fruit fly (*Ceratitis capitata* Wied.): attractants from angelica seed oil (*Angelica archangelica* L.). J. Chem. Ecol. 20: 1969–1984.

Flath, R. A., R. T. Cunningham, T. R. Mon and **J. O. John.** 1994b. Male lures for Mediterranean fruit fly (*Ceratitis capitata* Wied.): structural analogues of a-copaene. J. Chem. Ecol. 20: 2595–2609.

Franz, G., P. Kerremans, P. Rendon, and **J. Hendrichs.** 1996. Development and application of genetic sexing systems for the Mediterranean fruit fly based on a temperature sensitive lethal, p. 185–191. *In* McPheron, B.A. and Steck, G. J., eds., Fruit fly pests: a world assessment of their biology and management. St. Lucie Press, Delray Beach, FL.

Gonzalez, A., C. Rossini, M. Eisner, and **T. Eisner.** 1999. Sexually transmitted chemical defense in a moth (*Utetheisa ornatrix*). Proc. Natl. Acad. Sci. (USA) 96: 5570–5574.

Hare, J. F. and **T. Eisner.** 1993. Pyrrolizidine alkaloid deters ant predators of *Utetheisa ornatrix* eggs: effects of alkaloid concentration, oxidation state, and prior exposure of ants to alkaloid-laden prey. Oecologia 96: 9–18.

Hughes, P. R. 1973. Effect of alpha-pinene exposure on trans-verbenol synthesis in *Dendroctonus ponderosae* Hopkins. Naturwissenschaften 60: 261–262.

Jaffe, K., P. Sanchez, H. Cerda, J. V. Hernandez, R. Jaffe, N. Urdaneta, G. Guerra, R. Martinez and **B. Miras.** 1993. Chemical ecology of the palm weevil *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae): attraction to host plants and to a male produced aggregation pheromone. J. Chem. Ecol. 19: 1703–1720.

Krassnoff, S. B. and **D. E. Dussourd.** 1989. Dihydropyrrolizidine attractants for arctiid moths that visit plants containing pyrrolizidine alkaloids. J. Chem. Ecol. 15: 47–60.

Lance, D. R., D. O. McInnis, P. Rendon, and **C. G. Jackson.** 2000. Courtship among sterile and wild *Ceratitis capitata* (Diptera: Tephritdae) in field cages in Hawaii and Guatemala. Ann. Entomol. Soc. Am. 93: 1179–1185.

Lofstedt, C., N. J. Vickers, W. L. Roelefs and **T. C. Baker.** 1989. Diet related courtship success in the Oriental fruit moth, *Grapholita molesta* (Tortricidae). Oikos 55: 402–408.

Nishida, R., O. Iwahashi and **K. H. Tan.** 1993. Accumulation of *Dendrobium superbum* (Orchidaceae) fragrance in the rectal glands by males of the melon fly, *Dacus cucurbitae*. J. Chem. Ecol. 19: 713–722.

Nishida, R., K. H. Tan, M. Serit, N. L. Lajis, A. M. Sukari, S. Takahashi and H. Fukami. 1988. Accumulation of phenylpropanoids in the rectal glands of males of the Oriental fruit fly, *Dacus dorsalis*. Experientia 44: 534–536.

Nishida, R., T. E. Shelly and **K. Y. Kaneshiro.** 1997. Acquisition of female-attracting fragrance from a Hawaiian lei flower, *Fagraea berteriana*, by males of the oriental fruit fly. J. Chem. Ecol. 23: 2275–2285.

Ni**shida, R., T. E. Shelly, T. S. Whittier** and **K. Y. Kaneshiro.** 2000. α-copaene, a potential rendez-

vous cue for the Mediterranean fruit fly, *Ceratitis capitata*? J. Chem. Ecol. 26: 87–100.

Papadopoulos, N. T., B. I. Katsoyannos, N. A. Kouloussis and **J. Hendrichs.** 2001. Effect of orange peel substances on mating competitiveness of male *Ceratitis capitata*. Entomol. Exp. Appl. 99: 253–261.

Pliske, T. E. 1975. Courtship behavior and use of chemical communication by males of certain species of ithomiine butterflies (Nymphalidae: Lepidoptera). Ann. Entomol. Soc. Am. 68: 935–942.

Shelly, T.E. 2002. Feeding on papaya flowers enhances mating competitiveness of male oriental fruit flies *Bactrocera dorsalis* (Diptera: Tephritidae). Proc. Hawaiian Entomol. Soc. 35: 41–47.

Shelly, T.E. 2001a. Feeding on methyl eugenol and puakenikeni flowers increases long-range, female attraction by male oriental fruit flies, *Bactrocera dorsalis* (Diptera: Tephritidae). Fla. Entomol. 84: 634–640.

Shelly, T. E. 2001b. Exposure to α-copaene and α-copaene-containing oils enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 94: 497–502.

Shelly, T.E. 2000. Rasberry ketone and the mating success of male melon flies (Diptera: Tephritidae). Proc. Hawaiian Entomol. Soc. 34: 163–167.

Shelly, T. E. and **E. M. Villalobos.** 2004. Host-plant influence on the mating success of male Mediterranean fruit flies: variable effects within and between individual plants. Anim. Behav. In press.

Shelly, T.E. and **D.O. McInnis.** 2001. Exposure to ginger root oil enhances mating success of irradiated, mass-reared males of Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 94: 1413–1418.

Shelly, T. E., C. Dang and **S. Kennelly.** 2004. Exposure to orange (*Citrus sinensis* L.) trees, fruits, and oil enhances mating success of male Mediterranean fruit flies (*Ceratitis capitata* (Wiedemann)). J. Insect Behav. 17: 303–315.

Tanaka, N., L. F. Steiner, K. Ohinata and **R. Okamoto.** 1969. Low-cost larval rearing medium for mass production of oriental and Mediterranean fruit flies. J. Econ. Entomol. 62: 967–968.