Exposure to Tea Tree Oil Enhances the Mating Success of Male Mediterranean Fruit Flies (Diptera: Tephritidae)

Authors: Shelly, Todd E., and Epsky, Nancy D.

Source: Florida Entomologist, 98(4) : 1127-1133

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.098.0417

Exposure to tea tree oil enhances the mating success of male Mediterranean fruit flies (Diptera: Tephritidae)

Todd E. Shelly[1,*] *and Nancy D. Epsky*[2]

Abstract

The aroma of various plant essential oils has been shown to enhance the mating competitiveness of males of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Laboratory observations revealed that male medflies show strong short-range (<10 cm) attraction to tea tree oil (TTO hereafter) derived from leaves of the Australian plant *Melaleuca alternifolia* (Maiden & Betch) Cheel (Myrtales: Melastomataceae). The present study was undertaken to i) compare the attractiveness of TTO with that of trimedlure (the male lure routinely used in detection surveys) in field and field cage tests and ii) assess the influence of TTO exposure on male mating success under conditions of varying dose, duration of post-exposure (i.e., pre-test) interval, and access (contact possible or not) to the TTO source. Results showed that TTO-baited traps captured 50% as many males as trimedlure-baited traps in field cages but only 8% as many males as trimedlure-baited traps in the open field. Males exposed to pure TTO or dilutions of 50% and 5% TTO in hexane had higher mating success than non-exposed control males in tests conducted 1 d after exposure. TTO-exposed males also had a mating advantage when tested 3 d after exposure and when physical contact with the TTO source was prevented. In an additional experiment, TTO exposure was found to enhance the mating competitiveness of mass-reared, sterile males in competition against wild males for copulations with wild females in tests conducted 1 or 3 d after exposure.

Key Words: *Ceratitis capitata*; plant–insect interaction; female choice; pheromone calling

Resumen

Se ha demostrado que el aroma de varios aceites esenciales de plantas mejora la competitividad de apareamiento de los machos de la mosca mediterránea de la fruta (mosca de la fruta, moscamed), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Observaciones de laboratorio revelaron que los machos de la moscamed muestran una atracción fuerte de corto alcance (<10 cm) hacia el aceite del árbol de té (ATT) derivado de hojas de la planta australiana *Melaleuca alternifolia (*Maiden & Betch) Cheel (Myrtales: Melastomataceae). Se realizó el presente estudio para i) comparar la atracción hacia el ATT con la de trimedlure (el señuelo para los machos utilizado habitualmente en los sondeos de detección) en las pruebas de campo y en jaulas en el campo y ii) evaluar la influencia de la exposición de ATT en el éxito de apareamiento de los machos bajo condiciones de varias dosis, duración del intervalo de pos-exposición (antes de la prueba) y el aceso (contacto posible o no) a la fuente de ATT. Los resultados mostraron que las trampas cebadas con ATT capturaron el 50% del número de machos que las trampas cebadas con trimedlure en jaulas de campo, pero sólo el 8% del número de machos que las trampas cebadas con trimedlure en el campo abierto. Los machos expuestos a ATT puro o diluciones de 50% y 5% de ATT en hexano tuvieron un mayor éxito de apareamiento que los machos no expuestos de control en las pruebas realizadas 1 d después de la exposición. Los machos expuestos al ATT también tenían una ventaja de apareamiento cuando fueron probados 3 dias después de la exposición y cuando se evitó el contacto físico con la fuente ATT. En un experimento adicional, se encontró que la exposición al ATT mejora la competitividad de apareamiento de los machos estériles criados en masa, en competencia con los machos silvestres para copular con hembras salvajes en las pruebas realizadas 1 o 3 días después de la exposición.

Palabras Clave: *Ceratitis capitata*; interacción planta-insecto; elección femenina; feromona de llamada

In many herbivorous insects, constituents of sex pheromones are obtained via ingestion of plant-borne chemicals (Reddy & Guerrero 2004). In the true fruit flies (Diptera: Tephritidae), males of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), incorporate metabolites of the plant compound methyl eugenol into their sex pheromone (Nishida et al. 1988), and males that feed on methyl eugenol–bearing plants produce a more attractive pheromone and have a mating advantage over males without access to such plants (Shelly 2000a). Although less extensive, data on the melon fly, *Bactrocera cucurbitae* (Coquillett), suggest a similar scenario for this species: Males that feed on raspberry ketone spend more time "pheromone calling" and obtain more copulations than males deprived of this plant compound (Shelly 2000b). More recently, ingestion of the plant compound zingerone (also known as vanillyacetone) has been shown to enhance the mating success of males of *Bactrocera tryoni* (Froggatt), the Queensland fruit fly (Kumaran et al. 2013).

Plant compounds also influence the mating behavior of males of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann). Males exposed to the fruits or leaves of various *Citrus* species (Sapindales: Rutaceae) (Papadopoulos et al. 2001; Shelly et al. 2004; Shelly 2009; Kouloussis et al. 2013) and the fruits or bark of common guava (*Psidium guajava* L.; Myrtales: Myrtaceae) (Shelly & Villalobos 2004) have a mating advantage over non-exposed males. Likewise, exposure to the essential oils of different *Citrus* species (Shelly et al. 2004; Shelly 2009; Kouloussis et al. 2013), manuka (*Leptospermum scopariu* [Forst. & Forst.]; Myrtales: Myrtaceae) (Shelly et al. 2008), and ginger (*Zingib-*

[1]United States Department of Agriculture, Animal and Plant Health Inspection Service, 41-650 Ahiki Street, Waimanalo, Hawaii 96795, USA
[2]Subtropical Horticultural Research Station, United States Department of Agriculture, Agricultural Research Service, 13601 Old Cutler Road, Miami, Florida 33158, USA
*Corresponding author; E-mail: todd.e.shelly@aphis.usda.gov

er officinale Roscoe; Zingiberales: Zingiberaceae) (Shelly 2001) have been shown to confer a male mating advantage. Presumably owing to the higher concentration of particular chemicals, exposure to the aroma of these oils alone was sufficient to boost mating success, whereas direct contact with citrus leaves and fruits was required for mating enhancement. Essential oils contain dozens of individual compounds, and, at present, only 2 plant compounds (the terpenes α-copaene and linalool) have been demonstrated to enhance (by themselves) the mating success of male medflies (Shelly 2001; Juan-Blasco et al. 2013). More recently, another compound α-humulene was shown to actually reduce pheromone-calling and mating success of male medflies (Shelly & Nishimoto 2015). Thus, although mating enhancement has more commonly been observed, it appears that certain plant compounds may adversely affect *C. capitata* males.

The goal of the present study was to determine whether exposure to tea tree oil (TTO hereafter), derived from leaves of the Australian plant *Melaleuca alternifolia* (Maiden & Betch) Cheel (Myrtales: Melastomataceae), influences the mating success of male medflies. This question was prompted by research on the attractiveness to medfly of volatile chemicals from various plant species (Niogret et al. 2011) and essential oils. Whereas captures in TTO-baited traps did not differ significantly from those in blank controls in field tests conducted in Honduras, TTO was as attractive as trimedlure (the standard male attractant used in detection surveys; Jang & Light 1996) to male medflies over short distances (<10 cm) in small cage bioassays (N.D.E., unpublished data). Here, we i) compared the relative attractiveness of TTO and trimedlure (hereafter TML) in the open field and in field cages and ii) assessed whether exposure to TTO influenced male mating success under conditions of varying dose, duration of post-exposure interval prior to testing, and accessibility to the TTO source (i.e., contact possible or prevented).

Materials and Methods

INSECTS

With the exception of 1 mating experiment, all flies used in the study were derived from a laboratory colony started with 500 to 600 adults reared from coffee, *Coffea arabica* L. (Gentianales: Rubiaceae), collected near Kalaheo, Kauai, Hawaii, USA. The colony was maintained in several screen cages (60 × 40 × 30 cm; approx. 500 flies per cage), and flies were provided with an ample food mixture of sugar and yeast hydrolysate at a 5:1 volumetric ratio, water, and oviposition substrate (perforated plastic vials containing small sponges soaked in orange juice). Eggs were placed on standard larval medium (Tanaka et al. 1969) in plastic trays positioned above a layer of vermiculite, where pupation occurred. Once sifted from the vermiculite, pupae were placed in screen cages for adult emergence. Adults were separated by sex within 2 d of emergence, well before reaching sexual maturity (males: 6–8 d of age; females: 8–10 d of age) and placed in cubical (30 cm per side) screen cages (approx. 300 flies per cage) with food and water. Flies were held at 23 to 27 °C and 50 to 80% RH under natural light with photoperiod of 11:13 h L:D. When used in the experiments, males were 8 to 12 d old, females were 10 to 14 d old, and in a given experiment control and treated individuals of the same sex were the same age. When used in this study, the flies were 4 to 8 generations removed from the wild and are hereafter referred to as wild-like flies.

To distinguish males of different treatments in the mating experiments, pupae from the wild-like strain were coated with fluorescent dye of different colors (blaze orange or horizon blue; DayGlo Corporation, Cleveland, Ohio, USA) at rate of 2.5 g per 1 L of pupae, following the standard procedure used in Sterile Insect Technique (SIT) programs against the medfly (Andress et al. 2012). Upon emergence, the flies generally retain dye particles on the body that can be viewed with a dissecting microscope under UV illumination (black light). However, where external dye was not conspicuous, the head was crushed with forceps to examine the collapsed ptilinum, which picks up dye particles upon adult emergence from the puparium.

In 1 mating experiment, we also used males from a mass-reared, genetic sexing strain (Vienna-7/Tol-99) produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility, Waimanalo, Oahu, Hawaii, USA. This temperature sensitive lethal (*tsl*) strain possesses a sex-linked mutation, such that exposing eggs to high temperature kills female zygotes, thereby allowing production of males exclusively (Franz et al. 1994). This particular strain is currently used in an ongoing SIT program in southern California, USA. Larvae of the mass-reared strain were reared on the same standard diet mentioned above. Two days before eclosion, pupae were dyed (neon red; DayGlo Corporation, Cleveland, Ohio, USA) and then irradiated under hypoxia at 150 Gy of gamma radiation from a ^{137}Cs source. Pupae were placed in cubical screen cages, and emerged flies were held under the same conditions as the wild-like flies. When tested, *tsl* males were 5 to 10 d old.

ATTRACTION

We compared the relative attractiveness of TTO and TML to wild-like male medflies in experiments conducted in the open field and in field tents. Field tests were performed in a commercial coffee plantation near Haleiwa, Oahu, Hawaii, USA, during Aug to Oct 2014, with plants occurring in parallel rows separated by 2 to 3 m. We identified 2 trapping stations (i.e., coffee plants) separated by approximately 100 m and used these same 2 stations for all replicates. On a given test day, we placed 1 Jackson trap (IAEA/FAO 2003) baited with TTO (Puritan's Pride Inc., Oakdale, New York, USA) at 1 station and 1 Jackson trap baited with TML (Farma Tech Inc., North Bend, Washington, USA) at the other station. For both chemicals, 1 mL of liquid was applied to a cotton wick held in a perforated basket suspended over the sticky floor of the trap. In addition, at each station we placed a "blank" Jackson trap approximately 1 m from the lure-baited trap that contained a sticky panel and a cotton wick without lure.

At each station, we released 75 male medflies at each of 4 sites 25 m from the trap (i.e., 300 males were released per station per replicate). Two of the 4 release sites were along the same row as the trap station, and the other 2 release sites were in adjacent rows along a line perpendicular to the direction of the row containing the trap. Flies were released between 10:00 and 11:00 AM, and traps were collected and flies scored 24 h later. Replicates were separated by 4 to 7 d, and the locations of the TTO and TML baits were alternated between stations between successive replicates. All males released on a given date were dyed the same color (orange or blue), and the dye color used was alternated between successive replicates. TTO and TML baits were discarded after each replicate, and new baits were prepared for all trials. Twelve replicates were conducted for this experiment. Based on readings for Haleiwa (AccuWeather.com), daily minimum and maximum temperatures varied between 19 and 24 °C and between 27 and 33 °C, respectively, over the trapping period.

Trials also were performed in field cages to compare short-term, close-range attraction of male medflies to TTO and TML. Two nylon mesh cages of the type regularly used for fruit fly mating tests (FAO/IAEA/USDA 2014; 3 m diameter, 2.5 m height) were erected outside our laboratory in Halawa, Oahu, Hawaii, USA. The field cages were placed 3 m apart in full sun, and each was covered by a shade cloth

and contained 3 potted guava plants, each approximately 2 m tall, that were placed in the center of the tent. On a given day, we placed a Jackson trap baited with TTO in one cage and a Jackson trap baited with TML in the other cage. Traps were placed 1.25 to 1.50 m above ground in the center of the guava canopy; the same trap sites were used in all replicates. For both chemicals, 1 mL of liquid was applied to a cotton wick as described above. No blank traps were used in the field cage tests. Just prior to the test, males were placed in transparent plastic containers (0.5 L volume) and then released by gently opening the containers, which were placed on the cage floor at maximum distance from the trap. One hundred flies were released per cage; flies exited the containers at their own volition. Flies were released between 10:00 and 10:30 AM, and the number of males captured on the trap's sticky floor was scored 2 h later. After a trial, the remaining (untrapped) flies were killed with a fly swatter. Trials were conducted on 10 different days in Jan to Feb 2015 with temperatures ranging from 24 to 27 °C. The TML- and TTO-baited traps were alternated between field cages in successive trials; lures were applied anew for each trial.

CHEMICAL EXPOSURE PRIOR TO MATING TRIALS

The same basic protocol was used to expose males to TTO in all experiments, and variations in this procedure are noted below for the individual mating experiments. Using an aspirator, we transferred 90 males to a cubical (30 cm per side) screen cage. We then introduced a glass Petri dish containing a cotton wick (3 cm long, 1 cm diameter) to which TTO or a solution containing TTO had been applied. Independent of the dose, TTO-laden wicks were introduced between 10:00 and 11:00 AM and removed after 1 or 3 h (see below), and food (the same sugar–yeast hydrolysate described above) and water were then supplied. Whereas 90 flies were placed in the individual cages, we used only 80 flies per cage in the mating trials, thus allowing for minor differences among cages in mortality. Cages containing treated (TTO-exposed) and control (not exposed to TTO) flies were held in separate rooms under the temperature and humidity conditions noted above.

MATING TESTS: WILD-LIKE FLIES EXCLUSIVELY

All mating trials were performed in the field cages described above and followed the same protocol. Males were released in the cages at 08:00 AM, 15 to 20 min prior to female release to allow the establishment of calling sites. Eighty treated males and 80 control males were released and competed for matings with 80 females. All males were dyed, with the dye color (orange or blue) alternated between treated and control males in successive trials. Cages were checked every 30 to 45 min (matings typically last >90 min; Eberhard 2000) until 12:00 PM, and mating pairs were collected by gently coaxing them into plastic vials. At the conclusion of a trial, the vials were placed in a freezer, and dye color of the males was later determined under a black light using a dissecting microscope. Mating trials were conducted in Jan to Apr 2015 with temperatures varying between 23 and 28 °C.

Three mating experiments were conducted using wild-like flies exclusively, and 12 replicates were completed for each (2 field cages per day on 6 separate days).

Experiment 1. Mating tests were conducted 1 d after exposure of treated males to various doses of TTO. In 3 separate sets of trials, treated males were exposed to pure TTO, a 50% (by volume) TTO–hexane solution, or a 5% TTO–hexane solution. For the pure and 50% TTO treatments, we applied 100 µL to a cotton wick, which was placed in the cage for 1 h. For the 5% TTO–hexane solution, we used 200 µL and exposed the males for 1 h.

Experiment 2. Treated males were exposed to a cotton wick containing 5% TTO–hexane solution (200 µL for 1 h as above), but in this experiment mating tests were conducted 3 or 5 d after exposure of the treated males.

Experiment 3. Treated males were exposed to a cotton wick containing 5% TTO–hexane solution (200 µL for 1 h as above), but in this experiment the wick was covered with nylon screen to prevent direct fly contact with the chemical. Mating tests were conducted 1 d after exposure of the treated males.

MATING TESTS: *TSL* MALES AND WILD-LIKE FLIES

Consistent with many other studies (see Eberhard 2000 for a review), we recently found that wild-like males outcompeted sterile males of the aforementioned mass-reared *tsl* strain for copulations with wild-like females. In trials using equal numbers of males of the 2 types, *tsl* males obtained on average only 31% of the matings per replicate (Shelly, unpublished data). Based on results obtained using other plant oils (e.g., Steiner et al. 2013), we conducted another experiment to assess whether TTO exposure might increase the mating competitiveness of *tsl* males.

Experiment 4. Trials were conducted in the same manner described above except that, on any given day, 1 field cage contained 80 wild-like males, 80 treated (exposed to 200 µL of 5% TTO–hexane solution on an uncovered wick for 1 h) sterile *tsl* males, and 80 wild-like females, whereas the other field cage contained 80 wild-like males, 80 control (non-exposed) sterile *tsl* males, and 80 wild-like females. Two sets of trials were conducted, with treated *tsl* males tested 1 or 3 d after exposure. For both of these intervals, we completed 12 replicates for treated and control *tsl* males (1 field cage per day per male type over 12 d), with the *tsl* treatments alternated between tents on successive test days.

MALE CALLING

As described in the Results, wild-like males provided TTO had higher mating success (with only one exception) regardless of the specific exposure protocol used. Consequently, we examined the possibility that this enhancement resulted, at least in part, from increased signaling activity. We placed 80 treated (200 µL of 5% TTO–hexane solution on uncovered wick for 1 h on the day prior to testing) and 80 control males in the field cages at 08:00 AM. After 1 h, we collected 30 pheromone-calling males (i.e., with abdomen curved upward and rectal epithelium everted, appearing as a "bubble"; Arita & Kaneshiro 1986) individually in vials, chilled the males, and then scored the presence/absence of dye; one male category was dyed per trial, with the category receiving dye alternated between successive test days. On a given day, this test was run simultaneously in the 2 field cages, and trials were conducted on 6 different days (N = 12 total replicates).

DATA ANALYSES

Male captures in TTO- and TML-baited traps were compared using a Mann–Whitney test for the field experiment and a *t*-test for the field cage experiment as the data did not meet parametric assumptions in the former instance but did so in the latter. Numbers of matings obtained by treated and control males were compared using the *t*-test exclusively as data met the parametric assumptions in all cases. Examination of possible day effects was precluded by the small number of replicates performed per test day (i.e., only 2 field cages per day), and the possibility of a cage effect was discounted as mating frequencies of treated and control males were similar in the 2 field cages. Means ± 1 SE are presented. Statistical analyses were performed using SigmaPlot 11.0.

Results

ATTRACTION

In the coffee field, released males were captured in both TML- and TTO-baited traps but in significantly greater numbers in the former. On average, 119.7 ± 5.2 males (or 39.9% of the total number released) were recorded for the TML-baited trap per replicate compared with only 9.8 ± 1.6 males (or 3.3% of the total number released) in the TTO-baited trap (T = 78.0, P < 0.001). No males were captured in any replicate in the blank trap placed near the TTO-baited trap, whereas low numbers (1.75 ± 0.9, range: 0–11) were trapped in the blank trap near the TML-baited trap. Captures in the baited traps were significantly greater than those in the blank trap for both TML-baited (T = 222.0, P < 0.001) and TTO-baited traps (T = 216.0, P < 0.001). The same trend was observed in the field cages although the relative difference in captures between the lures was much smaller. On average, 46.5 ± 2.9 males were captured in the TML-baited trap per replicate compared with only 23.2 ± 2.6 males in the TTO-baited trap (t = 5.9, P < 0.001).

MATING TESTS: WILD-LIKE MALES

Experiment 1. In tests conducted 1 d after TTO exposure, treated males displayed a mating advantage for the 3 TTO concentrations used (Fig. 1). The relative mating success of TTO-exposed males was consistent across the treatments: Over the 3 concentrations, the treated males obtained 56 to 59% of the total matings recorded per replicate ($F_{2, 33}$ = 0.3, P = 0.69; ANOVA on arcsine transformed percentages).

Experiment 2. In tests conducted 3 d after TTO exposure, treated males achieved significantly more matings than control males (23.1 ± 0.9 vs. 17.8 ± 2.0, respectively; Fig. 2). In contrast, when tested 5 d after TTO exposure, treated and control males obtained similar numbers of matings per replicate (18.8 ± 0.7 vs. 19.7 ± 1.3, respectively; Fig. 2).

Experiment 3. In tests conducted 1 d after TTO exposure where contact with the cotton wick was prevented, treated males had significantly higher mating frequency than control males (28.5 ± 1.4 vs. 21.1 ± 1.2, respectively; t = 3.0, P = 0.006).

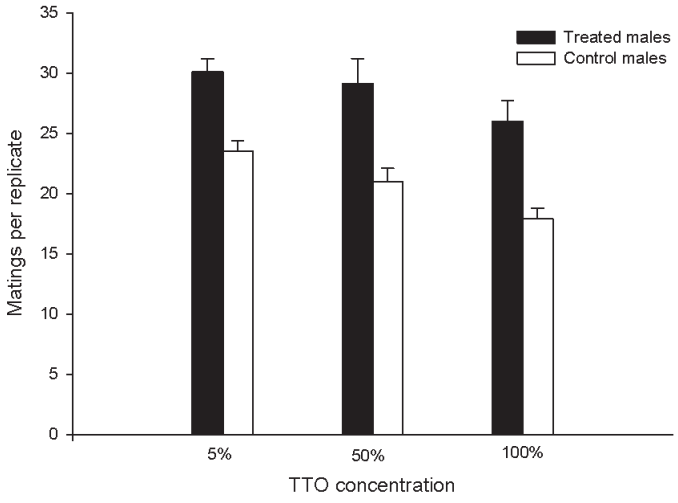

Fig. 1. Number of matings (mean ± SE) obtained per replicate by wild-like medfly males exposed to TTO (treated) or not exposed (control) for the 3 different TTO concentrations provided to the treated males (the 5% and 50% doses represent TTO concentration [v/v] in a hexane solution). Bar heights represent averages; whiskers represent 1 SE. Results of t-tests: 5% solution: t = 4.8, P < 0.001; 50% solution: t = 3.4, P = 0.003; pure (100%): t = 3.8, P < 0.001; N = 12 replicates at each concentration.

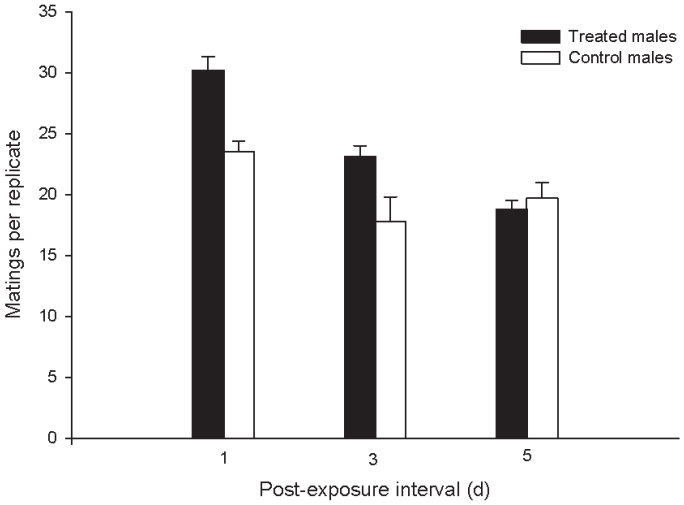

Fig. 2. Number of matings (mean ± SE) obtained per replicate by wild-like medfly males exposed to TTO (treated) or not exposed (control) for 3 different post-exposure (pre-test) intervals for the treated males (exposed to 200 µL of 5% TTO on an uncovered wick for 1 h). Bar heights represent averages; whiskers represent 1 SE. Results of t-tests: 1 d: t = 4.8, P < 0.001; 3 d: t = 2.4, P = 0.03; 5 d: t = 0.6, P = 0.53.

MATING TESTS: *TSL* MALES AND WILD-LIKE FLIES

Experiment 4. In tests conducted 1 d after TTO exposure (200 µL of 5% TTO–hexane solution on an uncovered wick for 1 h), treated *tsl* males accounted for roughly the same number of matings per replicate as wild-like males (21.7 ± 1.4 vs. 24.9 ± 2.3, respectively), whereas non-exposed *tsl* males achieved significantly fewer matings per replicate than wild-like males (14.3 ± 1.2 vs. 28.4 ± 1.7, respectively; Fig. 3A). On average, TTO-exposed males accounted for 47% of total matings per replicate compared with only 33% for non-exposed males (t = 3.5, P = 0.002). In tests conducted 3 d after exposure, wild-like males obtained significantly more matings per replicate than either TTO-exposed *tsl* males (29.6 ± 1.6 vs. 17.6 ± 0.7, respectively) or non-exposed *tsl* males (27.7 ± 1.5 vs. 12.2 ± 1.6, respectively; Fig. 3B). TTO-treated *tsl* males accounted for a greater proportion of the total matings per replicate than non-exposed *tsl* males (37 vs. 30%, respectively), although this difference was not statistically significant (arcsine transformed proportions, t = 1.8, P = 0.08).

MALE CALLING

Exposure to TTO (200 µL of 5% TTO–hexane solution for 1 h on the day prior to testing) appeared to increase pheromone-calling in wild-like males. On average, 17.4 ± 0.7 of the 30 calling males (58%) collected per trial were TTO-treated males, and 12.6 ± 0.6 were non-exposed control males (t = 5.1, P < 0.001).

Discussion

A mark-release-recapture experiment conducted in a Hawaiian coffee field revealed that TTO-baited traps were attractive, albeit only slightly, to male medflies. On average, TTO-baited traps captured approximately 10 of 300 males released per trial, a value significantly greater than that observed for nearby blank controls. As noted in the

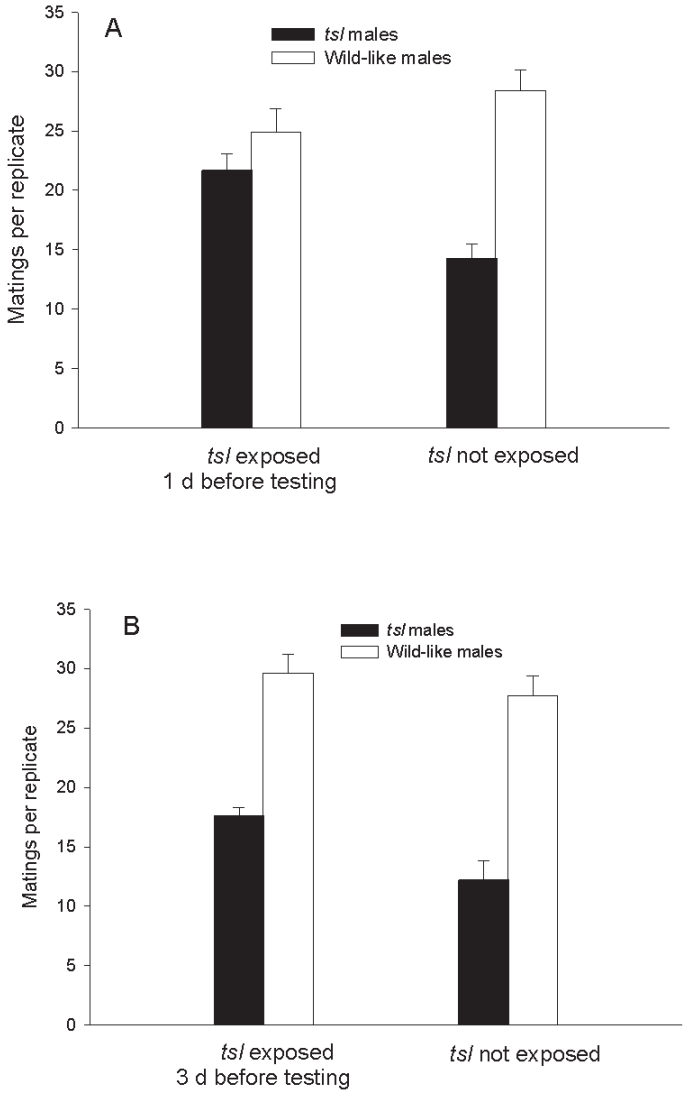

Fig. 3. Number of matings obtained per replicate by *tsl* males and wild-like males where *tsl* males were or were not exposed to TTO and tested either 1 d (A) or 3 d (B) after exposure. Wild-like males were not exposed to the chemical in any trial. Bar heights represent averages; whiskers represent 1 SE. Results of *t*-tests: (A) 1 d post-exposure interval: treated *tsl* males $t = 1.2$, $P = 0.24$; non-exposed *tsl* males $t = 6.8$, $P < 0.001$; (B) 3 d post-exposure interval: treated *tsl* males $t = 7.4$, $P < 0.001$; non-exposed *tsl* males $t = 6.6$, $P < 0.001$.

Introduction, previous work found that traps baited with TTO were no more attractive than unbaited traps to wild male medflies in a field study conducted in Honduras (N.D.E., unpublished data). The discrepancy between the 2 studies most likely reflects differing methods. In the present study, a relatively large number of males (300) was released relatively close (25 m) to a trap, resulting in a relatively high capture rate. In the study in Honduras, on the other hand, captures in the more attractive TML-baited traps averaged only 37 males per week, which suggests only a small population of wild medflies existed at the test site. Thus, the poor performance of the TTO-baited traps in that study perhaps reflected the combination of low TTO attractiveness and a small male population. Both studies were conducted in coffee fields, indicating that habitat differences probably did not account for the differing performance of traps containing TTO.

Not surprisingly, a greater proportion of medfly males were captured in baited traps in field cages compared with the field. In relative terms, nearly 8 times as many males, on average, were captured in the TTO-baited traps in field cage as in open field trials (23 vs. 3%, respectively). The difference was much less pronounced for TML-baited traps, in which only 15% more males, on average, were captured in the field cages than in the open field (46 vs. 40%, respectively). As a result, the difference in capture rates between TML- and TTO-baited traps was much smaller for the field cage trials (46 vs. 23% = 2-fold difference) compared with the open field (40 vs. 3% ≈ 13-fold difference). The results are not directly comparable, because different sampling intervals were used in the field cages (2 h) and the open field (24 h), but they nonetheless suggest, consistent with the previously mentioned findings, that TTO is only weakly attractive to male medflies over long distances (e.g., >10 m) but moderately to highly attractive over short distances (e.g., <3 m).

Essential oils from various plant species have been shown to enhance the mating success of male medflies (Shelly 2001; Shelly et al. 2008; Kouloussis et al. 2013), and, based on the present study, TTO should now be included in this group. The nature of the TTO-mediated mating enhancement was largely similar to that observed for other oils:

i. Increased mating success: wild or wild-like males. Although the methods varied among studies, it appears that, in mating competition between oil-exposed and non-exposed wild or wild-like males for same-type females, the advantage observed for TTO-exposed males (56–59% of total matings, experiment 1) was relatively low compared with other oils. For example, males exposed to orange oil accounted for 57 to 70% of all matings over several studies (Shelly et al. 2004; Papadopoulos et al. 2006; Kouloussis et al. 2013). Similarly, the data of Shelly (2001) and Papadopoulos et al. (2006) showed that males exposed to ginger root oil achieved 59 to 81% of all matings.

ii. Increased mating success: mass-reared males. Exposing mass-reared, sterile males to ginger root oil invariably resulted in increased mating competitiveness relative to wild or wild-like males. However, the degree to which this exposure boosts mating success varied, with mass-reared males exposed to ginger root oil being inferior competitors (but with improved performance over non-exposed, mass-reared males), equal competitors, or even superior competitors to wild or wild-like males (Shelly & McInnis 2001; Shelly et al. 2002). The present study demonstrated that TTO-exposed sterile males were equivalent to wild-like males in competition for copulations with wild-like females.

iii. Duration of exposure period. Most studies employed exposure periods longer than the 1 h interval used in the present study, but studies with ginger root oil (Shelly 2001) and manuka oil (Shelly et al. 2008) also reported heightened mating success of wild-like medfly males following only 1 h of exposure.

iv. Method of presentation. Consistent with studies involving ginger root oil (Shelly 2001) or orange oil (Shelly et al. 2004), wild-like males exposed to a covered TTO source, and thus prevented from contacting it, still displayed a mating advantage over non-exposed males.

v. Post-exposure duration of mating advantage. Although scant data are available, the effects of ginger root oil or orange oil exposure on male mating success appeared longer lasting than that of TTO exposure. Wild-like males exposed to ginger root oil had a mating advantage as long as 8 to 10 d after exposure (Shelly 2001), and increased male mating success was reported 5 d after exposure to orange oil (Shelly et al. 2004). By comparison, a mating boost was observed 3 d after TTO exposure but not 5 d after exposure. Results with mass-reared, sterile males appear similar. Mass-reared sterile males tested 5 d after exposure to ginger root oil

displayed the same mating success relative to wild-like males as sterile males tested 1 d after exposure to ginger root oil (Shelly & McInnis 2001). With TTO, however, *tsl* males tested 3 d after exposure were less competitive relative to wild-like males than *tsl* males tested 1 d after TTO exposure.

vi. Effect on male sexual signaling. In the present study, wild-like males exposed to TTO were more abundant in collections of pheromone-calling individuals than non-exposed control males. This result is consistent with data showing that wild-like males exposed to orange oil or ginger root oil spent more time signaling than non-exposed males (Papadopoulos et al. 2006). Other studies (Shelly 2001; Papadopoulos et al. 2006) indicated that oil exposure had little or no effect on the attractiveness of the male pheromone to females, but this remains untested for TTO-exposed males.

As noted in the Introduction, plant oils contain large numbers of compounds, and identifying which of these affect(s) the behavior of male medflies is a difficult task, particularly because behavioral effects may represent the combined action of multiple compounds. Although not addressing this problem directly, the present study is notable, because TTO apparently lacks the sesquiterpene α-copaene (Swords & Hunter 1978; Butcher et al. 1994; Keszei et al. 2010), which has been implicated as a key compound underlying enhanced mating performance of male medflies (Shelly 2001; Mavraganis et al. 2008). Although this obviously does not negate the role of α-copaene, it clearly indicates that other plant compounds may influence medfly sexual behavior. This interpretation parallels recent findings regarding medfly attraction to different genotypes of avocado *Persea americana* Mill. (Laurales: Lauraceae), which showed that, despite the strong attractiveness of α-copaene to medfly males (Flath et al. 1994a,b), no correlation was detected between male attraction and the amount of α-copaene present across these different genotypes (Niogret et al. 2011). Whereas this result may have reflected the occurrence of different enantiomeric forms across genotypes, the authors also suggested that additional sesquiterpenes may influence male medfly response. Although the impact of plant-borne chemicals on medfly behavior remains largely unstudied, it appears future work will broaden beyond the earlier focus on α-copaene to include a wider array of compounds. Regarding TTO, several terpinenes, most notably terpinen-4-ol, are major constituents (Swords & Hunter 1978; Butcher et al. 1994; Keszei et al. 2010), and the behavioral response of male medflies to these compounds merits investigation.

Acknowledgments

We thank Jon Nishimoto for capable assistance in maintaining the flies and performing mating trials.

References Cited

Andress E, Jones E, War M, Shelly T. 2012. Effects of pre-release chilling on the flight ability of sterile males of the Mediterranean fruit fly (Diptera: Tephritidae). Florida Entomologist 95: 587-592.

Arita LH, Kaneshiro KY. 1986. Structure and function of the rectal epithelium and anal glands during mating behavior in the Mediterranean fruit fly. Proceedings of the Hawaiian Entomological Society 26: 27-30.

Butcher PA, Doran JC, Slee MU. 1994. Intraspecific variation in leaf oils of *Melaleuca alternifolia* (Myrtaceae). Biochemical Systematics and Ecology 22: 419-430.

Eberhard WG. 2000. Sexual behavior and sexual selection in the Mediterranean fruit fly, *Ceratitis capitata* (Dacinae: Ceratitidini), pp. 459-489 *In* Aluja M, Norrbom AL [eds.], Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, Florida, USA.

FAO/IAEA/USDA (Food and Agriculture Organization, International Atomic Energy Agency, and United States Department of Agriculture). 2014. Product Quality Control for Sterile Mass-reared and Released Tephritid Fruit Flies. Version 6.0. International Atomic Energy Agency, Vienna, Austria.

Flath RA, Cunningham RT, Mon TR, John JO. 1994a. Additional male Mediterranean fruit fly (*Ceratitis capitata* Wied.) attractants from angelica seed oil (*Angelica archangelica* L.). Journal of Chemical Ecology 20: 1969-1984.

Flath RA, Cunningham RT, Mon TR, John JO. 1994b. Male lures for Mediterranean fruit fly (*Ceratitis capitata* Wied.): structural analogs of α-copaene. Journal of Chemical Ecology 20: 2595-2609.

Franz G, Gencheva E, Kerremans P. 1994. Improved stability of sex-separation strains for the Mediterranean fruit fly, *Ceratitis capitata*. Genome 37: 72-82.

IAEA/FAO (International Atomic Energy Agency and Food and Agriculture Organization) 2003. Trapping guidelines for area-wide fruit fly programmes. International Atomic Energy Agency, Vienna, Austria.

Jang EB, Light DM. 1996. Olfactory semiochemicals of tephritids, pp. 73-90 *In* McPheron BA, Steck GJ [eds.], Fruit Fly Pests: A World Assessment of their Biology and Management. St. Lucie Press, Delray Beach, Florida, USA.

Juan-Blasco M, San Andrés V, Martinez-Utrillas MA, Argilés R, Pla I, Urbaneja A, Sabater-Muñoz B. 2013. Alternatives to ginger root oil aromatherapy for improved mating performance of sterile *Ceratitis capitata* (Diptera: Tephritidae) males. Journal of Applied Entomology 137 (Suppl. 1): 244-251.

Keszei A, Hassan Y, Foley WJ. 2010. A biochemical interpretation of terpene chemotypes in *Melaleuca alternifolia*. Journal of Chemical Ecology 36: 652-661.

Kouloussis NA, Katsoyannos BI, Papadopoulos NT, Ioannou CS, Iliadis IV. 2013. Enhanced mating competitiveness of *Ceratitis capitata* males following exposure to citrus compounds. Journal of Applied Entomology 137 (Suppl. 1): 30-38.

Kumaran N, Balagawi S, Schutze MK, Clarke AR. 2013. Evolution of lure response in tephritid fruit flies: phytochemicals as drivers of sexual selection. Animal Behaviour 85: 781-789.

Mavraganis VG, Liaropoulos C, Papadopoulos NT, Kouloussis NA, Broumas T. 2008. Whole body extract of Mediterranean fruit fly males elicits high attraction in virgin females. Entomologia Experimentalis et Applicata 127: 20-29.

Niogret J, Montgomery WS, Kendra PE, Heath RR, Epsky ND. 2011. Attraction and electroantennogram responses of male Mediterranean fruit fly to volatile chemicals from *Persea*, *Litchi*, and *Ficus* wood. Journal of Chemical Ecology 37: 483-491.

Nishida R, Tan KH, Serit M, Lajis NL, Sukari AM, Takahashi S, Fukami H. 1988. Accumulation of phenylpropanoids in the rectal glands of males of the oriental fruit fly, *Dacus dorsalis*. Experientia 44: 534-536.

Papadopoulos NT, Katsoyannos BI, Kouloussis NA, Hendrichs J. 2001. Effect of orange peel substances on mating competitiveness of male *Ceratitis capitata*. Entomologia Experimentalis et Applicata 99: 253-261.

Papadopoulos NT, Shelly TE, Niyazi N, Jang E. 2006. Olfactory and behavioral mechanisms underlying enhanced mating competitiveness following exposure to ginger root oil and orange oil in males of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). Journal of Insect Behavior 19: 403-418.

Reddy GVP, Guerrero A. 2004. Interactions of insect pheromones and plant semiochemicals. Trends in Plant Science 9: 256-260.

Shelly TE. 2000a. Flower-feeding affects mating performance in male oriental fruit flies *Bactrocera dorsalis*. Ecological Entomology 25: 109-114.

Shelly TE. 2000b. Effects of raspberry ketone on the mating success of male melon flies (Diptera: Tephritidae). Proceedings of the Hawaiian Entomological Society 34: 143-147.

Shelly TE. 2001. Exposure to α-copaene and α-copaene–containing oils enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae). Annals of the Entomological Society of America 94: 497-502.

Shelly TE. 2009. Exposure to grapefruits and grapefruit oil increases male mating success in the Mediterranean fruit fly (Diptera: Tephritidae). Proceedings of the Hawaiian Entomological Society 41: 31-36.

Shelly TE, McInnis DO. 2001. Exposure to ginger root oil enhances mating success of irradiated, mass-reared males of Mediterranean fruit fly. Journal of Economic Entomology 94: 1413-1418.

Shelly TE, Nishimoto JI. 2015. Exposure to the plant compound α-humulene reduces mating success in male Mediterranean fruit flies (Diptera: Tephritidae). Annals of the Entomological Society of America: in press. DOI: 10.1093/aesa/sav008.

Shelly TE, Villalobos EM. 2004. Host plant influence on the mating success of male Mediterranean fruit flies: variable effects within and between individual plants. Animal Behaviour 68: 417-426.

Shelly TE, Robinson AS, Cáceres C, Wornoayporn V, Islam A. 2002. Exposure to ginger root oil enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae) from a genetic sexing strain. Florida Entomologist 85: 440-445.

Shelly TE, Dang C, Kennelly S. 2004. Exposure to orange (*Citrus sinensis* L.) trees, fruit, and oil enhances mating success of male Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]). Journal of Insect Behavior 17: 303-315.

Shelly TE, Cowan AN, Edu J, Pahio E. 2008. Mating success of male Mediterranean fruit flies following exposure to two sources of α-copaene, manuka oil and mango. Florida Entomologist 91: 9-15.

Steiner E, Woods W, McInnis DO, Lindsey J, Fogliani R, Soopaya R. 2013. Ginger root oil increases mating competitiveness of sterile Mediterranean fruit fly (Diptera: Tephritidae) in Western Australia. Journal of Applied Entomology 137 (Suppl. 1): 103-112.

Swords G, Hunter GLK. 1978. Composition of Australian tea tree oil (*Melaleuca alternifolia*). Journal of Agricultural and Food Chemistry 26: 734-737.

Tanaka N, Steiner LF, Ohinata K, Okamoto R. 1969. Low-cost larval rearing medium for mass production of Oriental and Mediterranean fruit flies. Journal of Economic Entomology 62: 967-968.