EXPOSING ENTIRE ADULT HOLDING ROOMS CONTAINING STERILE MALE MEDITERRANEAN FRUIT FLIES TO ORANGE OIL INCREASES THE MATING SUCCESS OF THOSE MALES IN FIELD-CAGE TRIALS

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ABSTRACT

A previous study demonstrated that exposing entire rooms holding mass-reared males of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), to the aroma of ginger root oil increased mating success relative to that observed for non-exposed males. Here, we followed the same experimental protocol to test whether the aroma of orange oil similarly enhanced male mating performance. Working at an eclosion facility in California, we exposed trailers (132 m^3) containing ≈ 14 million *C. capitata* males from a genetic sexing (*tsl*, temperature sensitive lethal) strain to orange oil aroma for 24 h and compared mating success of non-exposed or ginger root oil-exposed *tsl* males in field-cage trials (in which *tsl* males competed against males from a standard, bisexual strain for females from this same standard strain). Both orange oil- and ginger root oil-exposed *tsl* males achieved a significantly greater proportion of the total matings than non-exposed (to any aroma) *tsl* males. No difference in relative mating success was observed between orange oil- and ginger root oil-exposed *tsl* males. As orange oil is considerably less expensive than ginger root oil, its use could reduce costs substantially.

Key Words: Tephritidae, *Ceratitis capitata*, sterile insect technique, orange oil, mating

RESUMEN

Un estudio previo demostró que al exponer cuartos enteros que tenían machos criados en masa de la mosca mediterránea de la fruta, *Ceratitis capitata* (Wied.) a la aroma de aceite de raíz de jengibre aumento el éxito de aparearse en relación con lo que fue observado en machos no expuestos. Aquí, nosotros seguimos el mismo protocolo experimental para probar si el aroma de aceite de naranja de modo parecido mejora el desempeño de los machos en el apareamiento. Trabajando en una facilidad de eclosión en California, nosotros expusimos traileres (132 m^3) que tenían 14 millón de machos de *C. capitata* de una cepa que separar los sexos genéticamente (s*tl*, sensible a la temperatura letal) al aroma de aceite de naranja por 24 horas y comparamos su éxito de aparearse con machos (*stl*) no expuestos o expuestos al aceite de jengibre en pruebas realizadas en jaulas en el campo (en cual los machos *stl* competieron con los machos de una cepa estándar bisexual para la hembras de la misma cepa). Los machos *stl* expuestos al aceite de naranja y los expuestos al aceite de la raíz de jengibre lograron a tener una proporción más alta de apareamientos totales que los machos *stl* no expuestos (al aroma). No hubo una diferencia en el éxito del apareamiento relativo observada entre los machos *stl* expuestos al aceite de naranja y los expuestos al aceite de la raíz de jengibre. Como el aceite de naranja es mucho menos caro que el aceite de la raíz de jengibre, su uso puede reducir los gastos substancialmente.

The success of the Sterile Insect Technique (SIT) depends, to a large degree, on the sexual competitiveness of the released, sterile insects. Thus, protocols that enhance this competitiveness will increase the effectiveness of SIT as smaller numbers of sterile insects may be needed to control incipient wild populations (thereby reducing programmatic costs). Numerous studies (Shelly 2001; Shelly et al. 2003; 2004a, 2005, 2006, 2007) have demonstrated that exposure of adult males of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), to the aroma of ginger root oil (GRO hereafter, *Zingiber officinale* Roscoe) increases their mating success over that observed for non-exposed males. These studies have, through time, involved GRO exposure to increasing numbers of males held in containers of increasing size. For example, in the initial study (Shelly 2001), GRO exposure involved groups of 25 males held in plastic drinking cups (400 mL), whereas in a recent study (Shelly et al. 2007) entire trailers (132 m^3 volume) holding ≈ 14 million males were exposed to GRO with evidence of enhanced mating performance in field-cage trials. Conducted in southern California, this latter study involved mass-reared, sterile males used in a preventative release program, and

based on the "positive" result obtained in the mating trials, the California program has since instituted GRO exposure as a component of the pre-release environment.

Here, we describe a second set of experiments conducted at the same California facility that tested the effectiveness of orange oil (OO hereafter, *Citrus sinensis* L.) in enhancing the mating success of mass-reared *C. capitata* males. Previous studies (Shelly et al. 2004b; Papadopoulos et al. 2006) demonstrated that exposing small groups of *C. capitata* males to OO resulted in a significant mating advantage over non-exposed males. As OO is an inexpensive alternative to GRO (see below), we undertook the present study to determine whether large-scale OO exposure was as effective as GRO exposure and thus represented a viable, cost-cutting substitute.

Materials and Methods

The methods closely followed Shelly et al. (2007), consequently only a brief outline is presented here. Work was conducted during Jul-Aug, 2006, at the USDA-CDFA eclosion facility, Los Alamitos, CA, which receives daily shipments from Guatemala of irradiated, dyed (fluorescent pink) pupae from a temperature sensitive lethal (*tsl*) genetic sexing strain. Upon arrival, the pupae (nearly all of which are male) are immediately placed in plastic boxes (Plastic Adult Rearing Containers or PARCs, with screen-covered openings for ventilation), and food (sucrose-agar gel) is placed on the screened opening on the top of the boxes, which are then stored in trailers, each holding ≈ 360 boxes or ≈ 14 million *tsl* males. Males emerge as adults 2 d later and are then released (after chilling to facilitate the procedure) into the environment 2 d after peak emergence.

Two experiments were conducted. First, we compared the mating success of *tsl* males exposed or not exposed to OO (Oil Orange Valencia C.P. FCC, Citrus and Allied Essences Ltd., Lake Success, NY). In the second experiment, we compared mating success among *tsl* males exposed to OO, exposed to GRO (Chinese Ginger FCC, Citrus and Allied Essences Ltd., Lake Success, NY), or not exposed to either aroma. To expose males, 9 mL of OO or GRO were placed at each of 4 locations per trailer for a total dose of 36 mL per trailer (132 m^3). The trailers contained fans for ventilation, and the oil was placed near fans to enhance dispersal of the aroma. The OO or GRO was introduced at 0600 h 1 d prior to scheduled chilling/release and removed 24 h later just before chilling. Control (no OO or GRO exposure) *tsl* males were collected from trailers that did not receive (and never had received) exposure to either oil.

To obtain oil-exposed and control *tsl* males for the mating trials, we randomly selected and marked storage boxes several days before chilling. Along with all other boxes in a trailer, the marked boxes were transferred to a refrigerated trailer for chilling at 4°C for 90 min. During 'knockdown' of the flies, we collected samples of 100-200 males from each box and placed them in screen-covered plastic buckets, which were subsequently held at 25°C for 2 or 3 d until testing (i.e., males were 4 or 5 d old when tested). Males from different treatment groups were held in separate rooms. All males were provided sugar-agar gel and water, and males from a given storage box were used only in a single mating trial (tent).

Because wild populations of *C. capitata* are absent from California and their importation for experimental purposes is prohibited, we used males and females from a standard, bisexual strain ('Maui') in the mating trials. Irradiated, dyed (fluorescent green) pupae were shipped 3 times per week from Hawaii. Adults from the Maui strain were separated by sex within 24 h of emergence and held isolated from (post-chilled) *tsl* males but under the same (post-chilling) conditions. Males from the Maui strain were fed sugar-agar gel, and females were fed a sucrose-yeast hydrolysate mixture (3:1, w/w). Maui flies were 5-8 d old when tested.

Mating trials were conducted in 4 nylon-mesh field tents (3 m diameter, 2.5 m high) set up in a vacant lot in full sun. For both experiments, we used 4 tents per test day. For the first experiment, OO-treated *tsl* males were tested in 2 tents, and control *tsl* males were tested in 2 tents on each of 4 test days (for a total of 8 replicates per treatment). For the second experiment, 3 tents were assigned to OO-treated *tsl* males, GRO-treated *tsl* males, and control *tsl* males, respectively, and on every test day, and the final tent contained a second replicate of 1 of the treatments. The treatment group tested in this 'extra' tent was alternated between successive test days. Trials were performed on 6 different days for the second experiment, yielding a total of 8 replicates per treatment.

In all trials, groups of 75 males from the Maui strain, 75 females from the Maui strain, and 75 *tsl* males (either OO-treated, GRO-treated, or control) were released in the tents between 0830-0900 h (males were released 15 min before females). Tents were covered with shade-cloth and contained 2 artificial trees (2 m tall with ≈ 450 leaves resembling those of *Ficus benjamina* L.). Mating pairs were collected for 4 h after release and chilled in a freezer. Males were then identified with an ultraviolet (black) light to determine dye color. Unmated flies were removed from the tents following completion of a trial. Assignment of *tsl* treatment groups to specific tents was alternated between successive test days. Air temperature ranged from 23-29°C during the tests.

Within- and between-experiment comparisons of the relative mating success (% total matings) of

tsl males in the different treatments were made by *t*-test or one-way ANOVA (with arc sine transformed proportions) followed by the Tukey test to identify statistically significant inter-pair differences.

RESULTS

In the first experiment, *tsl* males exposed to OO obtained a significantly greater proportion of the total matings per replicate than control *tsl* males (t = 5.2, df = 14, P < 0.001; Fig. 1). In the second experiment, there was significant variation in relative mating success among the 3 treatments of *tsl* males ($F_{2, 21}$ = 34.9, P < 0.001), with OO- and GRO-exposed males accounting (in proportional terms) for approximately twice as many matings as the control males (Fig. 1), but there was no significant difference in the relative mating success of OO- and GRO-exposed *tsl* males.

Comparisons between the 2 experiments revealed consistency in the mating patterns. Control *tsl* males displayed similar levels of mating success in both experiments (t = 1.74, df = 14, P = 0.11) as did the OO-exposed males (t = 1.95, df = 14, P = 0.07).

DISCUSSION

The present study revealed that exposing entire holding rooms to the aroma of OO enhanced the mating success of mass-reared males of *C. capitata*. We purposefully used the same method of exposure that was employed successfully for GRO (Shelly et al. 2007) and did not, owing to limited resources, test the efficacy of alternative exposure protocols. In our prior study (Shelly et al. 2007), we observed variable effects of GRO exposure with total dose. For example, a total dose of 18 mL GRO (distributed throughout the room in the same manner as the 36 mL dose) had no effect on male mating performance. Whether or not a similar dose-dependent relationship holds for OO is unknown and deserves future study.

The present study also demonstrated that, at a total dose of 36 mL per trailer, OO was equivalent to GRO in enhancing the mating competitiveness of *tsl* males. As OO is less expensive than GRO ($54/kg versus $94/kg, respectively, L. Milack, personal communication), use of OO could substantially reduce costs associated with pre-release aroma exposure. However, additional studies should be performed before release programs consider switching from GRO to OO. Most importantly, whole room exposure involving GRO conferred a mating advantage for as long as 5 d after exposure (Shelly et al. 2007). While OO-exposure to small groups of males (25 individuals in 400 mL containers) produced a similar result (Shelly et al. 2004b), it is not yet known whether whole room exposure with OO will likewise have long-lasting effects on male mating success, although Papadopoulos et al. (2001) reported that males exposed to wounded citrus fruits had a mating advantage lasting at least 12 d after exposure. In addition, GRO-exposure was found to have no negative impact on male survival under field conditions (Shelly et al. 2004a). Although it seems unlikely that OO-exposure would reduce male survival, data are not yet available to assess this possibility.

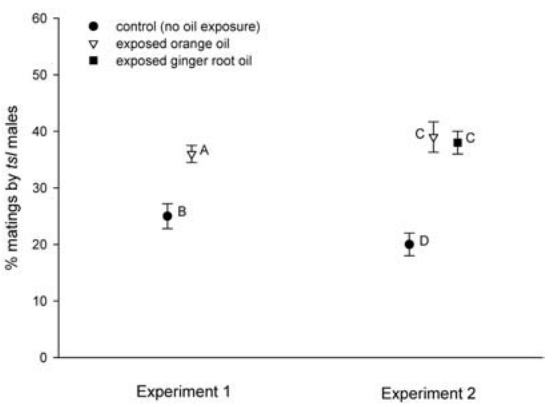

Fig. 1. Relative mating success of control (no oil exposure) and treated (orange oil- or ginger root oil-exposed) *tsl* males of *Ceratitis capitata* in field-cage mating trials. Values on ordinate represent the mean (±SE, n = 8) percentage of total matings obtained by *tsl* males per replicate. An average of 31-37 total matings were recorded per replicate over all treatments in the 2 experiments. Letters refer to statistical comparisons among means within experiments, and shared letters represent no significant difference (P = 0.05).

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