STERILE MALES OF THE MEDITERRANEAN FRUIT FLY EXPOSED TO GINGER ROOT OIL INDUCE FEMALE REMATING: IMPLICATIONS FOR THE STERILE INSECT TECHNIQUE (DIPTERA: TEPHRITIDAE)

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The Sterile Insect Technique (SIT) is used worldwide to control infestations of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Hendrichs et al. 2002). The effectiveness of the SIT is constrained, in part, by artificial selection inherent in the mass rearing process, which leads to changes in male courtship behavior. This, in turn, reduces the sexual competitiveness of mass-reared males (Cayol 2000). There is also evidence (Bloem et al. 1993) that sterile males may not fully inhibit female remating, presumably owing to the reduced transfer of sperm and accessory gland products during copulation. This has serious implications for SIT programs because in *C. capitata* the final male partner fertilizes the majority of the eggs in multiply mated females (Saul & McCombs 1993). If an initial mating with a sterile male does not dramatically inhibit female receptivity, the mated female may remate with a wild male, whose sperm then fertilizes most of the eggs. Conversely, if a female mates initially with a wild male, her receptivity may be so dramatically reduced that any remating (possibly with a sterile male) will be unlikely.

Several recent studies (e.g., Shelly et al. 2004) have shown that the aroma of ginger root oil (GRO) enhances the mating success of mass-reared, sterile males of *C. capitata*. However, these studies did not consider possible effects of male GRO exposure on female remating. Here, we present data addressing two questions: (1) Are females that initially mated with wild males more likely to remate with GRO-exposed, sterile males than GRO-deprived, sterile males? (2) Do initial matings with GRO-exposed, sterile males reduce the likelihood of female remating with wild males?

Methods used to maintain flies and run remating trials closely follow earlier studies (Shelly & Kennelly 2002; Shelly et al. 2004), consequently only an abbreviated description of the experimental protocol is provided here. Owing to the limited availability of wild flies, we used flies from a laboratory colony started with >500 adults reared from field-collected coffee berries (these are termed "laboratory" flies hereafter). Adults were held in screen cages and provided a sugar-protein mixture, water, and an oviposition substrate. Eggs were placed on larval medium over vermiculite for pupation. Adults used in the experiments were separated by sex before reaching sexual maturity and kept in screen-covered buckets with food and water. When used, laboratory flies were 10-12 generations removed from the wild.

Mass-reared flies were from a temperature sensitive lethal (*tsl*) strain produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility, Waimanalo, Oahu. Larvae of the *tsl* mass-reared strain were reared on standard diet, and males were irradiated as pupae 2 d before eclosion. Irradiated pupae were placed in screen-covered plastic buckets, and the adult males were maintained on sugar agar gel under the same conditions as laboratory flies.

For GRO exposure, 100 *tsl* males were placed in a plastic bucket, and 80 μl of GRO were applied to a filter paper disk, which was then placed on the bottom of the bucket. This dose was similar (in terms of ml GRO/m^3 of the holding container) to a 1 ml application on a PARC box (the standard type used in SIT programs), found previously to enhance the mating success of *tsl* males (Shelly et al. 2004). For all tests, treated *tsl* males were exposed to GRO in an isolated room for 3 h when 4-5 d old and were used 2 d after exposure; control *tsl* and laboratory males were not exposed to GRO.

Four experiments were performed. Laboratory females were mated first to laboratory males and 2 d later were given the opportunity to remate with control (Experiment A) or treated (Experiment B) *tsl* males. In the second pair of experiments, laboratory females were mated first to control (Experiment C) or treated (Experiment D) *tsl* males and 2 d later were given the opportunity to remate with laboratory males. In all cases, laboratory females were 7-9 d old when first mated, and laboratory and *tsl* males were 7-11 and 5-8 d old, respectively, when used for mating.

Initial matings by females were obtained in the laboratory in plexiglass cages (30 × 30 × 40 cm). Approximately 200 laboratory females and 200 males of a given type were used per cage, and mating pairs were collected continuously for 2-3 h. Only females from pairs coupled for >90 min were tested for remating; males were discarded. Prior to the remating trials, mated females were provided with the sugar-protein mixture and water but no oviposition substrate. A complete set of remating experiments (A-D) was run indoors using the plexiglass cages and outdoors in field tents each containing 2 artificial trees. Fifty mated females and 50 males of a given type were placed in either cages or tents at 0800 hours, and mating pairs were collected over the next 4 h. The experiments were conducted in a randomized order.

In both the laboratory and field cages, females initially mated to laboratory males were more

TABLE 1. FREQUENCY OF FEMALE REMATING. VALUES REPRESENT MEAN NUMBER (± 1 SD) OF FEMALES REMATING PER REPLICATE. SEVEN AND 14 REPLICATES WERE CONDUCTED PER EXPERIMENT FOR THE LABORATORY AND FIELD TENT TRIALS, RESPECTIVELY. VALUES NOT SHARING ANY LETTER ARE SIGNIFICANTLY DIFFERENT (TUKEY'S TEST FOLLOWING DETECTION OF SIGNIFICANT VARIATION IN 1-WAY ANOVA WITH RAW DATA; α = 0.05 IN ALL CASES).

	Male Type		
Experiment	First Mating	Second Mating	Female Rematings
Laboratory cages			
A	Laboratory	Control *tsl*	8.6 (3.4)[a]
B	Laboratory	Treated *tsl*	16.4 (3.3)[b]
C	Control *tsl*	Laboratory	12.6 (5.1)[ab]
D	Treated *tsl*	Laboratory	7.8 (2.8)[a]
Field tents			
A	Laboratory	Control *tsl*	2.5 (3.0)[a]
B	Laboratory	Treated *tsl*	4.9 (2.1)[b]
C	Control *tsl*	Laboratory	4.2 (2.0)[ab]
D	Treated *tsl*	Laboratory	3.7 (1.6)[ab]

likely to remate with treated *tsl* males than control *tsl* males (Table 1). However, in both indoor and outdoor tests, the probability of female remating with a laboratory male was independent of the treatment status of the *tsl* male from the initial mating. There was no difference (indoor or outdoor) in remating between females first mated to a laboratory male and then offered control *tsl* males and females tested in the reverse sequence. Overall experiments, the incidence of female remating was much greater in the laboratory cages than the field tents, presumably reflecting the higher fly density in the smaller, laboratory cages.

Prior studies (e.g., Shelly et al. 2004) have demonstrated GRO exposure may improve SIT effectiveness by increasing the mating competitiveness of *tsl* males. The present findings indicate another potential benefit of GRO exposure: inducement of female remating with *tsl* males, which may, given the last male advantage in sperm competition, lower the reproductive potential of wild medfly populations.

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