Ability of Sterile Males to Inhibit Female Remating in the Melon Fly Zeugodacus cucurbitae (Diptera: Tephritidae)

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Ability of sterile males to inhibit female remating in the melon fly *Zeugodacus cucurbitae* (Diptera: Tephritidae)

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The sterile insect technique (SIT) is widely used to suppress or eradicate populations of pestiferous fruit flies (Diptera: Tephritidae). Success of the sterile insect technique depends largely on the ability of irradiated, sterile males to compete successfully against wild males to obtain copulations with wild females, which result in unviable eggs and population decline. Also, female tephritids typically have reduced receptivity for a certain time after mating, and ideally sterile males should inhibit female remating to a degree similar to that effected by wild males. Various studies have assessed whether female remating frequency varies with the fertility status of the initial mate, but these often involve only flies from laboratory colonies and do not include wild flies (e.g., Katiyar & Ramirez 1970). In fact, relatively few studies have mated wild females with either wild males or mass-reared, sterile males, and then measured the remating tendency of females when subsequently offered wild males. When adopted, this protocol has yielded differing results among tephritid species. For example, wild females of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) that first mated with mass-reared, sterile males remated at higher rates than wild females first mated to wild males (Vera et al. 2003), whereas the remating frequency of wild females of *Anastrepha ludens* (Loew) varied independently of the origin (laboratory vs. wild) or fertility status of the initial mate (Meza et al. 2014; Arredondo et al. 2017).

Moreover, with few exceptions (Katiyar & Ramirez 1970; Chapman et al. 1998; Pérez-Staples et al. 2008; Gavriel et al. 2009; Abraham et al. 2011a), female remating has been measured within 1 to 3 d of the initial mating, even though females of some species may live for several wk or even mo (e.g., Dhillon et al. 2005). As a result, there are few data that assess long-term, temporal changes in female receptivity after an initial mating and subsequent oviposition. Moreover, 2 of the studies cited above (Katiyar & Ramirez 1970; Chapman et al. 1998) involved laboratory strains.

The melon fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), is an important pest of cucurbit crops throughout the Pacific, Asia, and Africa (Dhillon et al. 2005). This species has been recorded from over 130 plant hosts from at least 30 families (McQuate et al. 2017). The sterile insect technique has been used to control *Z. cucurbitae*, most notably in Japan (Koyama et al. 2004). In the context of evaluating the sexual performance of sterile males, 2 prior studies investigated the frequency of female remating in *Z. cucurbitae*. Kuba and Itô (1993) reported that wild females showed similar levels of remating (when offered wild males as second mates), regardless of whether the initial mating involved a wild or mass-reared male. However, these authors measured female remating at only 1 interval (3 d) after the initial mating, did not allow females to oviposit after the initial mating, and used non-irradiated (i.e., fertile) males from a bisexual, mass-reared strain. Haq et al. (2013) investigated female remating using males of the same genetic sexing strain employed in the recent study (see below), but in this case females were presented with males of the same type (wild or laboratory) for both initial and repeat matings. Thus, the crucial data for sterile insect technique (degree of female mating inhibition when encountering wild males after mating initially with a sterile male) were not collected. In addition, as with Kuba and Itô (1993), female remating was scored at only 1 short interval (3 d) after the initial mating.

This study examined remating by wild females of the melon fly, *Zeugodacus cucurbitae* (Coquillett), that mated with a wild male or a sterile male, had subsequent opportunity to oviposit, and then were presented with wild males at 1, 10, or 20 d intervals after the initial mating.

Wild flies were obtained from a recently established laboratory colony started with 300 to 400 adults that emerged from zucchini (*Cucurbita pepo* L.) (Cucurbitaceae) collected near Kapolei, Oahu, Hawaii. Rearing protocol followed Shelly (2018). When tested, flies from this colony were 2 generations removed from the wild. Adult flies were separated by sex within 3 to 4 d of emergence and maintained on standard adult diet (3:1 mixture of sugar and protein hydrolysate; Angel Yeast Co., Tianjin, China). When first mated, wild flies were 18 to 25 d old. The genetic sexing strain (labelled T1) has been mass-reared for approximately 15 yr following standard procedures (Vargas 1989). Sterile males from this strain were shown to be effective in a pilot sterile insect technique program (McInnis et al. 2004, 2007). Two d before eclosion, pupae were irradiated with a gamma irradiator at 100 Gy (McInnis et al. 2004). Adult males were collected within 2 to 3 d of emergence, provided the same sugar/protein hydrolysate diet as the wild flies, and mated when 12 to 16 d old (T1 flies mature more rapidly than wild flies; McInnis et al. 2007). T1 females were not used in this study. All rearing and mating tests were conducted at 24 to 26 °C, 50 to 70% RH, and 13:11 h (L:D) photoperiod with natural and artificial light.

Mated wild females were obtained by placing 20 to 30 females and 30 to 40 wild or sterile males in plexiglass cages (40 cm L × 30 cm W × 30 cm H) with a sleeved opening in late afternoon (2–3 h before the dusk mating period). Copulating pairs were gently coaxed into vials and held overnight (mating typically lasts until dawn). Pairs were monitored for 2 h after dusk, and those that broke apart during this monitoring period were discarded. The following morning, mated females were placed in screen cages (30 × 30 × 30 cm; 20–30 females per cage) along with food and water, as well as zucchini slices for oviposition.

Mated females were offered an opportunity to remate with a wild male at 1, 10, or 20 d after their initial mating. For these remating trials, 10 females mated to a given male type were placed with 10 wild males in the plexiglass cages 2 to 3 h before sunset, and total matings were scored per cage 30 min after sunset. Six cages were observed per night,

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1 for each of the 3 remating intervals for females mated initially to a wild or sterile male (i.e., 3 time intervals × 2 initial male mating types = 6 cages per night). Remating was monitored on 8 separate nights, yielding N = 8 cages for each remating interval for females first mated to wild or sterile males. For females tested at 10 or 20 d, zucchini slices were placed in the cage for 8 h on alternate days. Note that individual females were tested only once for remating, an approach that insures statistical independence of the data collected.

As raw counts of female rematings per cage were normally distributed, a 2-way ANOVA was used for analysis and revealed that both male type ($F_{1, 42}$ = 216; P < 0.001) and time interval since initial mating ($F_{2, 42}$ = 17.1; P = 0.002) had significant effects on female remating tendency (Fig. 1). The interaction between these variables was not significant ($F_{2, 42}$ = 1.0; P = 0.38). With the effect of interval held constant, female remating was found to be significantly higher following an initial mating with a sterile male than a wild male (P < 0.001; Holms-Šidák test). The same multiple comparisons test revealed that, independent of the identity (sterile or wild) of the first mating partner, female remating levels differed significantly between 1 and 20 d after the initial mating (P < 0.001), but not between 1 and 10 d or between 10 and 20 d (P > 0.05 in both cases).

A recent manual (FAO/IAEA/USDA 2014) on quality control of mass-reared, sterile males used in fruit fly sterile insect technique specifies acceptable levels of performance for certain parameters (e.g., flight ability, mating competitiveness). However, there are no such specifications regarding the ability of sterile males to inhibit female remating. In the present study, female remating levels recorded at 1, 10, or 20 d after the initial mating were approximately 3.0, 2.4, and 1.4× higher for females first mated to sterile as opposed to wild males. Comparative data are scant, but at a 1 d interval wild females of *C. capitata* first mated to sterile males were about twice as likely to remate with wild males as were females first mated to wild males (Mossinson & Yuval 2003). Higher remating also was observed for *A. serpentina* (Wiedemann) females first mated to sterile males (Landeta-Escamilla et al. 2016), whereas remating levels did not differ between females of *A. fraterculus* (Wiedemann) females first mated to wild or sterile males (Abraham et al. 2013).

The factors influencing receptivity and remating in female tephritids appear to vary among tephritid species. In *C. capitata*, for example, depletion of stored sperm appears to promote female remating (Mossinson & Yuval 2003; Abraham et al. 2011b). In contrast, Kuba and Itô (1993) demonstrated that the presence and amount of sperm transferred to *Z. cucurbitae* females has no effect on their remating tendency. Thus, it appears that a substance(s) in the seminal fluid influences mating refractoriness of *Z. cucurbitae* females, and that this component is less abundant or less effective in T1 males than wild males. Improving the ability of T1 males to lessen female remating tendency may be challenging, as it may require both identifying the active substance and developing means (genetic, physiological, etc.) to render it more effective.

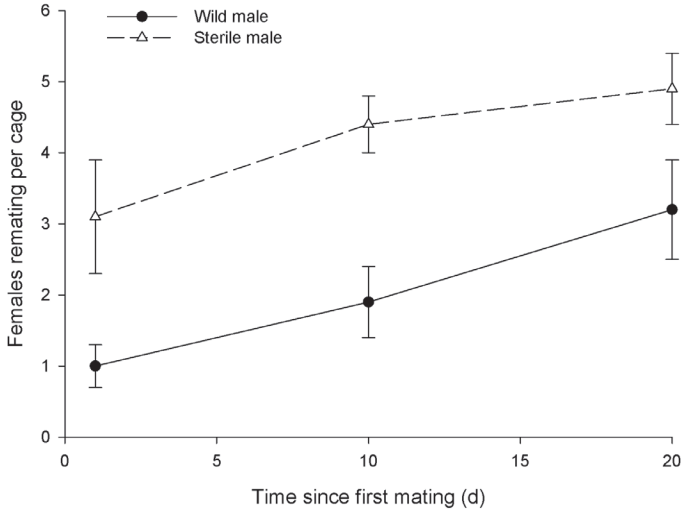

Fig. 1. Numbers of rematings observed per cage for females first mated to wild or sterile males at 3 intervals after the initial mating. Each cage held 10 test females. Symbols represent mean values ± 1 SE; N = 8 in all cases.

Summary

This study investigated the ability of mass-reared, sterile males of a genetic sexing strain to inhibit remating by wild females in the melon fly *Zeugodacus cucurbitae*. Virgin wild females were initially mated to either virgin wild or sterile males, and then given the opportunity to remate with wild males at 1, 10, or 20 d after the initial mating. Two-way ANOVA revealed that both male type and time since initial mating significantly influenced female remating levels. Initial matings with wild males resulted in lower female remating than initial matings with sterile males. Female remating levels increased with time elapsed since the initial mating, regardless of whether the first mate was a wild or sterile male.

Key Words: sterile insect technique; fruit fly; female remating; refractory period

Sumario

Este estudio investigó la capacidad de los machos estériles criados en masa de una cepa genética sexual para inhibir el reapareamiento de hembras silvestres de la mosca del melón *Zeugodacus cucurbitae*. Las hembras vírgenes silvestres se aparearon inicialmente con machos vírgenes silvestres o estériles, y luego se les dio la oportunidad de reaparearse con machos silvestres a 1, 10 o 20 días después del apareamiento inicial. El ANOVA de dos vías reveló que tanto la clase de macho como el tiempo desde el apareamiento inicial influyeron significativamente en los niveles del reapareamiento de las hembras. Los apareamientos iniciales con machos silvestres resultaron en menos reapareamientos en las hembras que los apareamientos iniciales con machos estériles. Los niveles de reapareamientos de las hembras aumentaron con el tiempo transcurrido desde el apareamiento inicial, independientemente de si el primer compañero era un macho silvestres o estéril.

Palabras Clave: técnica de insecto estéril; mosca de la fruta; reapareamientos de hembras; periodo refractario

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