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MATING COMPETITIVENESS OF MASS-REARED MALES OF THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE) FROM ECLOSION TOWERS

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ABSTRACT

In Florida, an ongoing Preventative Release Program utilizes the sterile insect technique to prevent infestations of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann). Unlike other such programs, which use plastic, storage (PARC) boxes, the Florida operation holds pupae and newly emerged adults in eclosion towers prior to release. Although eclosion towers save space and labor, few data exist regarding the quality of sterile male medflies held in towers versus PARC boxes. Here, we present the results of field-cage trials comparing the mating success of sterile males held in towers versus PARC boxes. In addition, previous research has shown that exposing PARC box-held males to the aroma of ginger root oil (GRO) increases their mating competitiveness. Consequently, we assessed whether a similar increase was evident for tower-held males. Finally, we performed a mark-release-recapture study involving GRO-exposed and non-exposed males and estimated their relative survival and dispersal in the field using the trap catch data. Data from the mating trials showed that sterile males held in towers displayed approximately the same mating success as sterile males held in PARC boxes and that, among tower-held males, GRO significantly increased mating competitiveness relative to non-exposed males. In the trapping study, significantly more GRO-exposed males were captured than non-exposed males, and there was no apparent difference in the duration of the post-release interval over which GRO-exposed and non-exposed males were captured. These findings, along with earlier comparisons of adult weight, flight ability, and yield suggest no obvious differences in the efficacy of tower and PARC-box eclosion systems for medfly sterile release programs.

Key Words: Mediterranean fruit fly, sterile insect technique, tower eclosion system

RESUMEN

En Florida, un Programa de Liberación Preventativa utiliza la técnica del insecto estéril para prevenir infestaciones de la mosca mediterránea de la fruta, *Ceratitis capitata* (Wiedemann). No como en otros programas que usan cajas plásticas de almacén (PARC), la operación en Florida se mantiene las pupas y adultos recién salidos en torres de eclosión antes de ser liberados. Aunque las torres de eclosión guarden espacio y requiren menos mano de obra, poca información existe en cuanto da la cualidad de los machos estériles de la mosca mediterránea de la fruta mantenidos en las torres versus los machos en las cajas PARC. Aquí, presentamos los resultados de las pruebas de jaulas en el campo comparando el éxito de apareamiento de los machos estériles mantenidos en torres versus los mantenidos en cajas PARC. Además, ha mostrado en investigaciones anteriores que al exponer machos mantenidos en las cajas PARC al aroma del aceite de la raiz de jengibre (ARJ) se aumentó su capacidad para competir en el apareamiento. Por lo tanto, nosotros evaluamos si habia un aumento similar evidente en los machos mantenidos en las torres. Por último, nosotros realizamos un estudio de marcar-liberar-recapturar con machos expuestos y no expuestos al ARJ y estimamos su sobrevivencia y dispersión relativa en el campo usando los datos del número de machos capturados en trampas. Los datos de las pruebas de apareamiento mostraron que los machos estériles mantenidos en torres tuvieron aproximadamente el mismo éxito de apareamento que los machos mantenidos en las cajas PARC y que, entre los machos mantenidos en las torres, el ARJ aumentó significativamente su capacidad para competir en el apareamiento en relación con los machos no expuestos al ARJ. En el estudio de atrapamiento, significativamente mas machos expuestos al ARJ fueron capturados que machos no expuestos al ARJ, y no hubo una diferencia aparente en la duración del intervalo pos-liberación cuando los machos expuestos y no expuestos al ARJ fueron capturados. Estos hallazgos, adjunto con las comparisiones anteriores del peso de adulto, habilidad con volar y rendimiento sugieren que no hay diferencias obvias en la eficiencia de los sistemas de eclosión de torres y cajas PARC para los programas de liberación de machos estériles de la mosca mediterránea de la fruta.

The Sterile Insect Technique (SIT) is widely used to suppress or eradicate infestations of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), a pest that attacks many commercially important fruits and vegetables worldwide (Hendrichs et al. 2002). Present SIT programs involve the production and sterilization of a large number of male pupae (in genetic sexing strains, a sex-linked, temperature sensitive lethal [*tsl*] mutation allows selective elimination of females in the egg stage, Franz et al. 1996), a prerelease holding period of 4-5 d during which pupae mature and adults eclose and feed, and aerial or ground release of the adult males into the environment. As even this brief outline suggests, the SIT is a relatively expensive management strategy both in terms of materials and labor, and consequently there is a persistent need to increase the efficiency of this protocol and reduce costs.

In 2002, the Florida Preventative Release Program against medfly began using a new system, the Tower Eclosion (TE) system, for emergence and feeding of adults prior to field release (Salvato et al. 2004). Each tower consists of interlocking, screen-paneled, aluminum frames or trays ($76 \times 76 \times 2.5$ cm, l:w:h) stacked on a wheeled (i.e., mobile) base. Pupae are placed in a trough around the edge of a tray, and food (a sugar-agar gelatin routinely used in medfly SIT) is placed on each screen panel. This procedure is repeated for each tray, and a completed tower consists of 60-80 pupal-holding trays. A small fan (blowing upwards) is fitted on the top of each tower for ventilation. Upon emergence, the flies move to the screen and feed, and the puparia are left behind in the trough. On the day of field release, towers are moved into a cold room, where the puparia are vacuumed from the troughs, and the trays are manually removed from the tower and turned upside down over a container to collect the chilled flies.

In Florida, the TE system replaced the Plastic Adult Rearing Container (PARC) system, which is still used in the ongoing medfly SIT programs in California and Guatemala. In the PARC system, pupae are placed in paper bags, which, in turn, are placed in plastic (PARC) boxes ($48 \times 60 \times 33$ cm, l:w:h) with screened panels on the lid and sides for ventilation. The sugar-agar gelatin is placed on the lid screen, and the boxes are stacked for storage. On the day of field release, boxes are moved into a cold room, bags are removed, and the boxes are turned upside down over a container to collect the chilled flies.

The TE system offers several advantages over the PARC system as follows: (1) the TE system requires much less space than the PARC system to hold a given number of flies, (2) the TE system reduces labor costs considerably, owing to automated pupal loading, puparia separation and disposal, and tray washing, and (3) by eliminating the use of paper bags, the TE system reduces supply costs, generates less waste, and reduces 'fly loss' (flies remaining inside the discarded paper bags) better than the PARC system. In addition to economic issues, however, a comprehensive comparison requires data on the performance of males held in the TE versus PARC systems. Potential economic benefits may become less compelling if, for some reason, males from the TE system are of poor quality relative to males from the PARC system. To date, only one study by Salvato et al. (2004), who found no significant differences in yield (emergence), adult weight, or flight ability between them, has compared the quality of medfly males from the two systems.

To provide further comparisons between the 2 holding systems, the present study, which was conducted in Hawaii and Florida, had 3 main objectives. First, in Hawaii we compared the mating success of sterile *tsl* males from eclosion towers or PARC boxes in competition with males from a recently established wild colony for females from that same colony. Second, in both Hawaii and Florida, we assessed the effectiveness of ginger root oil (GRO) in enhancing the mating performance of sterile *tsl* males held in eclosion towers. Prior work (Shelly 2001) has shown that exposure to the aroma of GRO significantly increases the mating performance of male medflies (a protocol termed 'aromatherapy'). Application of GRO to individual PARC boxes has already been shown to enhance male mating success (Shelly et al. 2004), and here we test for a similar effect with eclosion towers. Third, in Florida we performed a release-recapture study comparing trap captures of GRO-exposed versus non-exposed sterile *tsl* males to identify potential differences in post-release dispersal and longevity. Previous studies (Shelly et al. 2004; Levy et al. 2005) have not detected any negative effect of GRO on survival of male medflies (except for a specific diet-related effect, Levy et al. 2005), but these were conducted in field or laboratory cages, and data are lacking from the open field. Similarly, GRO exposure had no apparent effect of male performance on a laboratory flight-mill (S. Opp, personal communication), but no data are available examining potential effects of GRO exposure on male dispersal in the field.

Materials and Methods

Study Insects and Mating Trials—Hawaii

In Hawaii, *tsl* males were from the Vienna-7/Tol-99 strain produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility (Waimanalo, Oahu, HI). This strain has been mass-reared at the USDA-Moscamed facility in El Pino, Guatemala, since 1999, and ≈1.25 million eggs from this facility were used to start the colony in Hawaii in 2001. Males

used in the current study were dyed fluorescent pink (DayGlo Color Corporation, Cleveland, OH) and irradiated as pupae 2 d before eclosion in hypoxia at 150 Gy of gamma irradiation from a ^{137}Cs source.

Owing to the low availability of wild flies, we used flies from a recently established colony (REC, 4 generations removed from the wild) in the mating trials. This colony was derived from 300-500 adults reared from coffee berries collected on the island of Kauai. Adults were held in screen cages and provided with a sugar-protein (yeast hydrolysate) mixture (3:1 by weight), water, and an oviposition substrate (perforated plastic vials containing small sponges soaked in lemon juice). Eggs were placed on standard larval diet (Tanaka et al. 1969) in plastic containers over vermiculite for pupation. Adults used in the mating trials were separated by sex within 24 h of eclosion, well before reaching sexual maturity at 5-8 d of age (T.E.S., unpublished data) and kept in screen-covered buckets (5-liter volume; 100-125 flies per bucket) with ample food (sugar-protein mixture) and water.

Five experiments were conducted in Hawaii. In experiment 1, *tsl* males from eclosion towers or PARC boxes competed against REC males for REC females. On a given day, *tsl* pupae from the same batch were placed in 2 towers and 2 PARC boxes on the day of irradiation. The towers and boxes were kept in the same room under the same environmental conditions (25-27°C, 60-85% RH, 12:12 L:D). Because the aforementioned rearing facility supports the ongoing SIT program in California, our allotment of pupae was insufficient to use fully loaded eclosion towers (1 tower holds ≈ 1.25 million pupae). Consequently, we placed pupae (350 mL, where 1 mL ≈60 pupae) in a single tray per tower and left all other trays empty. For all trials, pupae were placed in the 30th tray from the bottom in towers built 60 trays high. A single slab of sugar agar gel of the same size used in the Florida program (15 × 9 × 3 cm, l:w:h) was placed on the pupae-containing trays. For each PARC box, we followed the protocol used in the California program and put 100 mL of pupae in each of 6 paper bags, which were then placed on the box floor, and placed a 20 × 15 × 3 cm (l:w:h) slab of the sugar agar gel on the screen panel on the box lid. Four d after pupal placement (i.e., 2 d post-peak emergence), flies were moved into a cold room (4°C for 10-15 min), and 100-200 flies from each tower tray or PARC box were transferred to a screen-covered, plastic bucket. These flies were provided sugar-agar gel and held at 25-28 °C until testing 2 d later (i.e., when most *tsl* males were 4 d old).

In experiments 2-5, *tsl* males from towers with or without GRO treatment competed against REC males for REC females. In each of these experiments, *tsl* pupae from the same batch were placed in 4 towers on the day of irradiation (as above, 350 mL of pupae were placed in the 30th tray of a given tower, and sugar agar was provided for food). Two towers were placed in each of 2 separate rooms under the same environmental conditions as above. In one room, the towers received no GRO exposure, and the flies (control males) were handled in the same manner described above. In the other room, we exposed each of the towers to GRO by applying 1 mL of GRO to a cotton wick (2.5 cm length, 1 cm diameter), placing the wick in an aluminum foil-lined Petri dish, and placing the Petri dish on the floor beneath the tower.

In experiments 2-4, GRO exposure commenced between 0800-0900 h on the day after peak emergence and continued for 24 h, at which time the flies (treated males) were chilled and collected. In experiment 2, the flies were tested 2 d after collection (i.e., as above, the majority of *tsl* males were 4 d old when tested), and in experiment 3, we assessed the long-term effectiveness of GRO exposure by holding the *tsl* males for 5 d before testing (i.e., control and treated *tsl* males were 7 d old when tested). In experiment 4, we followed the same protocol as experiment 2, except that we attempted to simulate fully-loaded towers by placing approximately 22,500 grains of rice ("flies"; ≈45 grains per mL; 500 mL used per tray) and a 15 × 9 cm piece of cardboard ("sugar agar") on all trays in the towers. This simulation was intended only to mimic the physical environment affecting the upper movement of air within towers, and it is recognized that odor absorption (possibly affecting GRO dispersion) may have differed between simulated and fully operational towers. Finally, in experiment 5, GRO was applied at the time of pupal loading and left until adults were chilled and collected (i.e., 4 d later). Trials were then conducted 2 d later (when *tsl* males were 4 d old).

Mating trials were conducted at the USDA-ARS-PBARC facility in Honolulu, Oahu, HI, during Apr-May, 2005. For the experiment comparing the mating success of *tsl* males held in towers versus PARC boxes, we released 75 REC males, 75 REC females, and either 75 tower-held *tsl* males or 75 PARC box-held *tsl* males in nylon-screen, field cages (diameter 3.0 m, height 2.5 m). For the experiments comparing the mating success of *tsl* males held in GRO-exposed towers versus non-exposed towers, we released 75 REC males, 75 REC females, and either 75 GRO-exposed *tsl* males or 75 non-exposed *tsl* males in field cages. When tested, REC males were 7-13 d old and REC females were 8-14 d old. REC males were not exposed to GRO in any trial. In both experiments, the *tsl* males released within a given field cage derived from the same tower or PARC box, and the *tsl* males from a given tower or PARC box were used in a single cage only (i.e., 4 tents were run per test day, with each containing *tsl* males from a single tower or PARC box). The field cages con-

tained 2 artificial trees (each 2 m tall with ≈450 leaves resembling those of *Ficus benjamina* L.) Artificial trees were used because they provided a chemically neutral substrate on which the flies display the entire complement of natural activities. Flies were released between 0800-0830 hours, mating pairs were collected over the following 4 h, and males were identified with a black light. Over 20% of females mated in all trials (the minimum proportion defining an acceptable trial, FAO/IAEA/USDA 2003), consequently no data were excluded. Air temperature ranged between 25-30 °C during the trials.

Study Insects and Mating Trials—Florida

In Florida, *tsl* males were from the same strain used in Hawaii, with pupae shipped directly by air from the Guatemalan rearing facility to the eclosion facility in Sarasota, FL. Prior to shipping, *tsl* pupae were dyed (fluorescent pink) and irradiated 2 d before eclosion in hypoxia at 145 Gy of gamma irradiation from a ^{60}Co source. Because wild (or REC) flies were unavailable, we used males and females from a standard, bisexual strain (Petapa) reared in Guatemala. Pupae from the Petapa strain also were dyed and irradiated before shipping, but a different dye color (green) was used for identification. Adults of the Petapa strain were separated by sex within 24 h of eclosion and held in transparent, plexiglass cages (40 × 30 × 30 cm, l:w:h; 300-500 flies per cage) with screen panels on the top and were provided sugar-agar gelatin as a source of food and water. Flies from the Petapa strain were 5-7 d old when tested.

In Florida, we conducted 3 mating experiments, all of which involved comparisons between non-exposed and GRO-treated males from eclosion towers, with GRO always applied at the time of pupal loading. In experiments 6 and 7, we applied 0.50 and 0.25 mL of GRO, respectively, to each of 10 filter paper squares (5 by 5 cm) and placed 1 square on trays 1 (bottom), 5, 10, 15, 20, 25, 30, 40, 50, and 60 (top). Thus, a total volume of 5.0 and 2.5 mL of GRO was used per tower in experiments 6 and 7, respectively. In experiment 8, we applied 1 mL of GRO to a cotton wick, placed the wick in a Petri dish, and placed the Petri dish on the bottom tray of the tower. In all 3 experiments, pupae and the sugar-agar gelatin were placed on all trays of a given tower (in the same amount as in Hawaii), except that trays holding GRO were left empty. GRO-exposed towers were kept in a separate room from non-exposed towers under the same environmental conditions (19-24°C, 60-80% RH). Peak emergence of adult males occurred 2 d and chilling occurred 4-5 d after pupal loading, and samples of males were taken from 4-6 trays from the middle of the tower (trays 20-40) during chilling. These males were transferred to cages, provided sugar agar gel, and held until testing 1-2 d later (i.e., *tsl* males were 3-5 d old when tested).

Mating trials were conducted at the eclosion facility in Sarasota, FL, during Feb-Apr, 2003, in the same manner as those in Hawaii except that (i) Petapa flies were used instead of REC (or wild) flies, (ii) *tsl* males from a given tower were used in 2 cages on each of 2 successive d (as in Hawaii, 4 cages - 2 with non-exposed and 2 with GRO-exposed *tsl* males - were run per day), (iii) the cages each contained a single potted ruby red grapefruit tree (*Citrus paradisi* M.), and (iv) tests were run between 1000-1400 h, owing to the relatively cool, winter temperatures (21-28°C).

Release-Recapture Study—Florida

The release-recapture study was conducted as part of the routine operation of the Florida Preventative Release Program. Test flies were released by air on 7 dates (at roughly 1-month intervals) between March-August, 2004, in 2 areas (Hillsborough and Sarasota Counties) included within the established trapping grid. On a given date, approximately 6 million (4 fully loaded towers) GRO-exposed or non-exposed *tsl* males were released in each area (on 1 date, GRO-exposed males were mistakenly released away from the target sites, resulting in a total of 7 releases for non-exposed males and 6 releases for GRO-exposed males; Table 3). Test flies were dyed green or blue to distinguish them from the pink-dyed flies released in the ongoing control program. To expose males, we applied 1 mL of GRO to a cotton wick placed in a Petri dish and placed the dish on the bottom tray of the tower at the time of pupal placement. GRO-exposed and non-exposed towers were kept in separate rooms under the same environmental conditions as above. Treatments as well as dye colors were alternated between the 2 test areas on successive releases; alternating colors allowed us to distinguish males from successive releases in the same area (because males were unlikely to survive ≥60 d, males from successive releases of the same color in a given area were unlikely to occur contemporaneously).

Releases were made from a small aircraft (Beechcraft BE90 King Air) flying at an approximate ground speed of 160 mph (250 km/h) at an altitude of 600-800 m in Hillsborough Co. and 800-1,100 m in Sarasota Co. Releases were made in 4 sites in Hillsborough Co. and 5 sites in Sarasota Co., where each site represented an east-west, oriented line along which 6 Jackson traps (baited with trimedlure) were placed at 264 m intervals for a total transect length of 1584 m (1 mile). Thus, the Hillsborough and Sarasota study areas contained 24 (4 sites, 6 traps per site) and 30 traps (5 sites, 6 traps per site), respectively. All sites were in residential areas, and traps were

typically placed 4-5 m above ground in citrus trees. During a release, the aircraft made 3 passes perpendicular to, and evenly spaced along, a site (trap line). Approximately equal numbers of flies were released per pass in each area. Traps were serviced daily, excluding weekends, and captured flies were examined under a black light.

Statistical Analyses

Comparisons of mating success were based on raw data with the *t*-test as the assumptions of normality and equal variance were met in all cases (excepting one instance, where data were $\log_{10}$ transformed to normalize the data). Proportions were likewise compared with the *t*-test but using arsine transformed values. Pairwise comparisons involving trapping data from the release-recapture study were made by using the Mann-Whitney test as data were not normally distributed. Preceding analyses were made with SigmaStat software (version 2.0). Slopes of regression lines showing temporal decline in male captures were compared following Zar (1996).

Results

Mating Trials—Hawaii

Table 1 presents the results of mating trials conducted in Hawaii. There was no significant difference found in the mating success of *tsl* males held in PARC boxes versus eclosion towers (experiment 1). On average, males from PARC boxes obtained 19% of all matings per replicate compared to 22% for males from towers ($P > 0.05$). The addition of GRO to eclosion towers after adult emergence (experiment 2) significantly increased the number of matings obtained by *tsl* males, with GRO-exposed males, on average, achieving over twice as many matings (20.1/8.8 = 2.3) per replicate as non-exposed *tsl* males. In this experiment, REC males had a mating advantage over both GRO-exposed and non-exposed, *tsl* males. However, the magnitude of this advantage varied with GRO treatment: on average, GRO-exposed males obtained 44% of all matings per replicate compared to only 20% for non-exposed males ($P < 0.001$). The effect of GRO was evident even 5 d after the exposure period (experiment 3). As before, GRO-exposed *tsl* males obtained, on average, twice as many matings (23.5/11.7 = 2.0) per replicate as non-exposed *tsl* males. In this experiment, REC males accounted for significantly more matings than non-exposed males, but no difference in mating frequency was found between REC males and the GRO-exposed males. Results from the full tower simulation (experiment 4) were similar to those obtained for the other experiments. GRO-exposed, *tsl* males obtained, on average, about 60% more matings (19.8/12.2 = 1.62) per replicate than non-exposed, *tsl* males, and there was no significant difference in mating frequency between REC and GRO-exposed, *tsl* males. GRO conferred a mating advantage even when applied at the time of pupal placement (experiment 5). In this case, GRO-exposed *tsl* males obtained significantly more matings than control *tsl* males and a similar number of matings as REC males. The timing of GRO application (post- or pre-adult

Table 1. Results of mating tests conducted in Hawaii. Values are means ±SE; differences between means were compared using the *t*-test. For a given experiment, lower case letters refer to comparisons between male types within a treatment group (i.e., within a row), and upper case letters refer to comparisons between treatment groups within a male type (i.e., within a column). For a particular comparison, means followed by different letters were different at $P = 0.05$. Sample sizes (n) refer to the number of field-cage tests run per treatment per experiment.

Experiment	n	Holding Unit	GRO[#]	Matings per replicate	
				tsl males	REC males
1	8	PARC box	—	8.0 ± 1.2 a, D	33.4 ± 3.2 b, E
		Tower	—	7.7 ± 1.4 c, D	27.4 ± 3.0 d, E
2	12	Tower	—	8.8 ± 1.2 a, D	35.6 ± 1.7 b, F
		Tower	+ (A/day 4)	20.1 ± 1.9 c, E	25.8 ± 1.7 d, G
3	8	Tower	—	11.7 ± 1.4 a, D	29.1 ± 3.2 b, F
		Tower	+ (A/day 7)	23.5 ± 2.3 c, E	23.1 ± 1.4 c, F
4	8	Tower	—	12.2 ± 2.1 a, D	29.3 ± 3.6 b, F
		Tower	+ (A/day 4, rice)	19.8 ± 2.4 c, E	24.0 ± 2.2 c, F
5	10	Tower	—	9.1 ± 1.3 a, D	28.1 ± 3.5 b, F
		Tower	+ (P/day 4)	19.2 + 2.1 c, E	20.8 + 2.8 c, F

[#]Control = No GRO (-), Treated = 1 mL GRO (+). A = GRO applied after adult emergence, P = GRO applied at time of pupal placement, day number = age of adult sterile males when tested, rice = rice placed on trays to simulate full tower.

emergence) did not affect the relative mating success of GRO-exposed males; the average proportion of total matings achieved by treated males was similar in experiments 1 (43%) and 5 (48%), respectively ($P > 0.05$).

Mating Trials—Florida

In Florida, application of GRO to eclosion towers at the time of pupal placement boosted the mating success of *tsl* males at all 3 doses tested (Table 2). Each of these experiments yielded the same basic results: (i) GRO-exposed, *tsl* males achieved significantly more matings per replicate than non-exposed, *tsl* males, (ii) Petapa males accounted for significantly more matings per replicate than non-exposed, *tsl* males, and (iii) Petapa and GRO-exposed, *tsl* males obtained similar numbers of matings per replicate.

Release-Recapture—Florida

Based on data pooled over both areas, GRO-exposed *tsl* males were captured in significantly greater numbers than non-exposed males ($t = 59.0$, $P < 0.05$, $n_1 = 7$, $n_2 = 6$, Mann-Whitney test; Table 3). On average, 298 GRO-exposed males were captured per release compared to only 76 non-exposed males. The small number of replicates precluded rigorous comparisons of the treatment groups within each of the release areas, but higher trap catches were noted for GRO-exposed than non-exposed males in both Hillsborough (1,018 versus 199, respectively) and Sarasota (772 versus 331, respectively).

In addition, we compared GRO-exposed and non-exposed *tsl* males with respect to the post-release interval over which males were trapped. Combining data from both release areas, we found no significant difference in the length of the 'capture interval' between GRO-exposed (mean = 16.7 d, range = 8 - 27 d) and non-exposed (mean = 11.3 d, range = 4 - 26 d) males ($t = 52.0$, $P > 0.05$, $n_1 = 7$, $n_2 = 6$, Mann-Whitney test).

The above results show that, although GRO-exposed males were trapped in higher numbers than non-exposed males, they were not trapped over substantially longer post-release intervals. Collectively, these findings suggest that the capture rate of GRO-exposed males declined more rapidly than for non-exposed males. However, closer inspection of daily trap captures reveals that numbers of GRO-exposed and non-exposed males declined at a similar rate in the day just after a release and that a steeper decline was evident for GRO-exposed males only later (Fig. 1). A $\log_{10}$ transform of the raw data yielded linear declines for both types of males, with nearly identical slopes between 1-10 d after release ($t = 0.2$, $P > 0.05$, $df = 16$). In contrast, the rate of decline in male captures from 11-30 d post-release was significantly greater for GRO-exposed males than non-exposed males ($t = 2.4$, $P < 0.05$, $df = 36$). This finding appeared to derive from the fact that nearly all (512/530 = 97%) of non-exposed males were captured in d 1-10 following release, resulting in a nearly horizontal line for capture rate over the later days. For GRO-exposed males, a larger proportion of trapped males were captured after d 10 (139/1,790 = 8%), with the temporal decline in their capture rate being more evident.

Because *tsl* males were released aerially over relatively large areas, the trapping data do not allow for rigorous comparison of the dispersal ability of GRO-exposed versus non-exposed *tsl* males. However, analysis of presence/absence data on a

TABLE 2. RESULTS OF MATING TESTS CONDUCTED IN FLORIDA. VALUES ARE MEANS ±SE; DIFFERENCES BETWEEN MEANS WERE COMPARED USING THE *t*-TEST. FOR A GIVEN EXPERIMENT, LOWER CASE LETTERS REFER TO COMPARISONS BETWEEN MALE TYPES WITHIN A TREATMENT GROUP (I.E., WITHIN A ROW), AND UPPER CASE LETTERS REFER TO COMPARISONS BETWEEN TREATMENT GROUPS WITHIN A MALE TYPE (I.E., WITHIN A COLUMN). FOR A PARTICULAR COMPARISON, MEANS FOLLOWED BY DIFFERENT LETTERS WERE SIGNIFICANTLY DIFFERENT AT $P = 0.05$. SAMPLE SIZES (n) REFER TO THE NUMBER OF FIELD-CAGE TESTS RUN PER TREATMENT PER EXPERIMENT.

Experiment	*n*	Holding Unit	GRO[#]	Matings per replicate	
				tsl males	REC males
6	8	Tower	—	4.0 ± 0.9 a, D	23.7 ± 2.7 b, F
		Tower	+ (5 mL)	12.6 ± 2.3 c, E	17.0 ± 2.6 c, F
7	10	Tower	—	10.3 ± 1.8 a, D	21.0 ± 2.5 b, F
		Tower	+ (2.5 mL)	20.0 ± 3.3 c, E	15.9 ± 2.4 d, G
8	10	Tower	—	8.0 ± 1.5 a, D	28.4 ± 7.1 b, F
		Tower	+ (1 mL)	17.7 + 2.3 c, E	19.5 + 4.4 c, G

[#]Control = No GRO (-), Treated = GRO applied at time of pupal placement (+). In all cases, sterile males were tested when 3-4 d old.

TABLE 3. TRAP CAPTURE OF GRO-EXPOSED AND NON-EXPOSED *tsl* MALES FOLLOWING AERIAL RELEASE. VALUES REPRESENT THE TOTAL NUMBER OF FLIES CAPTURED IN JACKSON TRAPS OVER ALL SITES IN THE 2 RELEASE AREAS, HILLSBOROUGH (H) AND SARASOTA (S). ON A GIVEN DATE, ONLY ONE TREATMENT GROUP (NON-EXPOSED OR GRO-EXPOSED) WAS RELEASED PER AREA, AND THE TREATMENT GROUP WAS ALTERNATED BETWEEN SITES OVER SUCCESSIVE DATES. ALL RELEASES WERE MADE IN 2004.

Release number	Release date	Number of trapped males	
		Non-exposed	GRO-exposed
1	3/2	105 (S)	391 (H)
2	3/30	74 (H)	573 (S)
3	4/28	45 (S)	416 (H)
4	5/25	54 (H)	96 (S)
5	6/29	134 (S)	[3] (H)#
6	7/27	71 (H)	103 (S)
7	8/31	47 (S)	211 (H)
Total		530	1,790

#Data were excluded from analysis, because flies were mistakenly released away from target sites.

trap-day basis suggests, preliminarily at least, that GRO-exposed and non-exposed males show similar dispersal. For the 1-week interval following a release, we scored the presence/absence of test flies over all traps and computed the proportion of total trap-days that were 'positive' (i.e., ≥1 test male present). Over all releases in both areas, GRO-exposed males were present in 47% of the trap-d compared to 19% for non-exposed males (*t* = 59.0, $P < 0.05$, $n_1 = 7$, $n_2 = 6$, Mann-Whitney test). The greater value observed for GRO-exposed was not unexpected given the higher number of GRO-exposed males captured overall. However, similar dispersal ability between the 2 male types is indicated by the fact that the relative difference in trap-day occurrence (47/19 = 2.5) between GRO-exposed and non-exposed males was similar to the relative difference in the total number of GRO-exposed versus non-exposed males recaptured in the week following releases (1506/495 = 3.0). In other words, relative abundance alone was a crude indicator of distribution independent of the GRO status of the males.

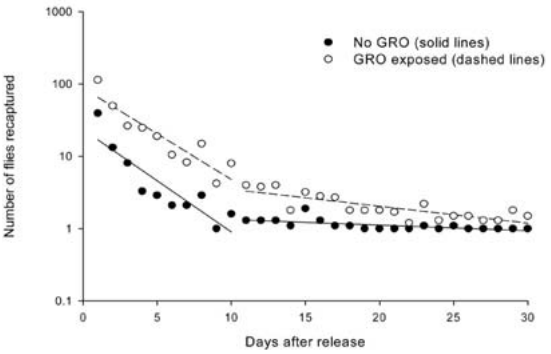

Fig. 1. Number of GRO-exposed and non-exposed *tsl* males captured on individual days following releases. Values represent average numbers (+1) computed over all releases; note ordinate is $\log_{10}$ scale. Simple linear regressions for $\log_{10}$ transformed male numbers were as follows: Non-exposed males, d 1-10: $Y = 1.37 - 0.14X$, $r^2 = 0.80$; Non-exposed males, d 11-30: $Y = 0.21 - 0.01X$, $r^2 = 0.43$; GRO males, d 1-10: $Y = 1.94 - 0.13X$, $r^2 = 0.91$; GRO d, 11-30: $Y = 0.75 - 0.02X$, $r^2 = 0.53$.

DISCUSSION

The present study shows that mating competitiveness, as measured in field-cage trials, is similar for sterile *tsl* males held in eclosion towers or PARC boxes. Along with the results of Salvato et al. (2004), which showed no difference in yield, weight, or flight ability, this finding indicates that there are no major differences in the overall quality of *tsl* males held in these 2 eclosion systems. Although longevity or dispersal ability have not yet been compared, the observed similarity in overall male vigor suggests that economic considerations, rather than concern over fly quality, will be the key determinant in programmatic decisions to switch from PARC to TE systems.

The present study also demonstrated a positive effect of GRO exposure on the mating performance of sterile, *tsl* males held in eclosion towers similar to that previously observed for males held in PARC boxes. For trials involving GRO application after adult emergence, the mating frequency of treated males (in competition with REC males for REC females) was approximately twice that observed for non-exposed males in both eclosion systems. In the PARC box system, GRO-exposed males (1 mL for 3 h) achieved 52% of the total matings compared to only 24% for non-exposed males (Shelly et al. 2004), while in the TE system, GRO-exposed males obtained 44% of the total matings compared to only 20% for the non-exposed males (experiment 2).

In contrast to PARC boxes, placement of GRO at the time pupal loading invariably boosted the mating success of *tsl* males held in eclosion towers. For PARC boxes, application of GRO at the time of pupal loading conferred a mating advantage to the subsequently emerged males for a dose of 1.0 mL but not for a dose of 0.25 mL (Shelly et al. 2004). Thus, for PARC boxes, a volumetric dose-to-container ratio of 0.0025 (0.00025 m^3 GRO/0.1 m^3 PARC box) did not result in increased male mating success. For tower-held males, however, a mating advantage was consistently observed following application of 1 mL GRO at pupal loading or at a dose-to-container ratio of only

0.0004 (0.001 m^3 GRO/2.5 m^3 eclosion tower) or 16% of the ineffective ratio noted above for PARC boxes. Why GRO, when applied at pupal placement, was more effective in towers than PARC boxes is not known. GRO exposure appears effective only when applied to adults: exposing pupae (but not adults) did not influence the mating performance of the subsequently emerged adults (Shelly 2001). Thus, the difference observed between PARC boxes and towers likely reflects a difference in the amount of GRO remaining in cotton wicks (placed under the towers; 3-dimensional dispenser with surface area of approximately 10 cm^2) as opposed to blotter paper (placed on PARC boxes; two dimensional dispenser with surface area of 25 cm^2). The difference could have also derived from differences in air circulation between the 2 holding systems. In PARC boxes, GRO was placed on a screened panel on the lid, and the odor was not directed downward into the box. In towers, on the other hand, GRO was placed beneath the towers, and its odor was drawn directly and continuously over the pupae and the emerged adults in the towers.

Consistent with earlier results (Shelly et al. 2004), the release-recapture study suggested there was no negative effect of GRO exposure on the survival of sterile males. GRO-exposed males were recaptured in significantly greater numbers and over similar post-release capture intervals as non-exposed males. As noted, the present study does not allow for a rigorous comparison of dispersal ability, but the analysis of trap-day occurrence suggests similar movement by GRO-exposed and non-exposed males. A planned field experiment in Hawaii will provide additional data on vagility by monitoring male captures at different distances from a central release point.

In conclusion, based on results from an ongoing study, it seems unlikely that the higher capture of GRO-exposed males in the Florida releases was an artifact of the GRO exposure itself. Preliminary field data from Hawaii indicate that males exposed to GRO in the laboratory are, in fact, less likely to be captured in trimedlure-baited traps in the field than naïve, non-exposed males. Thus, it does not appear that exposure in the laboratory to one attractant (GRO) increases male responsiveness to another lure (trimedlure). This finding, if validated, suggests that the Florida trapping data actually provide a conservative estimate of the relative abundance of GRO-exposed males in the environment.

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