Comparative lure response, dispersal, and survival of male melon flies (Diptera: Tephritidae) from wild and genetic sexing strains in Hawaii

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Abstract

The Sterile Insect Technique is an important component of area-wide programs to control invading or established populations of pestiferous tephritids. The sterile insect technique involves the release of large numbers of mass-reared, sterilized males to achieve sterile male × wild female matings, which yield infertile eggs and thus suppress the pest population. The development of male-only strains (also termed genetic sexing strains) has resulted in more effective control of wild populations than standard bisexual releases. A genetic sexing strain based on sex-linked pupal color exists for *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), an important agricultural pest worldwide, but how this strain might perform in a sterile insect technique program has not been thoroughly investigated. As documented for several tephritid species, artificial selection imposed via mass-rearing, particularly over long periods, may have negative effects on various biological parameters of the released flies, including flight ability and dispersion, life span, and mating competitiveness. The goal of the present study was to compare lure responsiveness, dispersal, and survival between males from genetic sexing strains and wild strains of *Z. cucurbitae*. Our results indicate that males of the 2 strains differed significantly in dispersal ability, but not in lure attraction or survival ability. The potential usefulness of the genetic sexing strains in sterile insect technique programs for control of *Z. cucurbitae* is assessed based on these findings.

Key Words: *Zeugodacus*; *cucurbitae*; Sterile Insect Technique; cue-lure; mark-release-recapture

Resumen

La técnica de los insectos estériles es un componente importante de los programas de área amplia para controlar las poblaciones invasoras o establecidas de tefrídas plagas. La técnica del insecto estéril implica la liberación de grandes cantidades de machos esterilizados criados en masa para lograr apareamientos estériles macho × hembra salvaje, que producen huevos infértiles y, por lo tanto, suprimen la población de la plaga. El desarrollo de cepas masculinas (también llamadas cepas de sexado genético) ha resultado en un control más efectivo de las poblaciones silvestres que las liberaciones bisexuales estándar. Existe una cepa genética de sexado basada en el color pupal vinculado al sexo para *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), una plaga agrícola importante en todo el mundo, pero no se ha investigado a fondo cómo podría funcionar esta cepa en un programa de técnica de insecto estéril. Según lo documentado para varias especies de tefritidas, la selección artificial impuesta mediante la cría en masa, particularmente durante largos períodos, puede tener efectos negativos en varios parámetros biológicos de las moscas liberadas, incluida la capacidad y dispersión del vuelo, la vida útil y la competitividad de apareamiento. El objetivo del presente estudio fue comparar la capacidad de respuesta del señuelo, la dispersión y la supervivencia entre machos de cepas genéticas de sexado y cepas salvajes de *Z. cucurbitae*. Nuestros resultados indican que los machos de las 2 cepas diferían significativamente en la capacidad de dispersión, pero no en la atracción de señuelos o la capacidad de sobrevivencia. En función de estos hallazgos, se evalúa la utilidad potencial de las cepas de sexado genético en los programas de técnicas de insectos estériles para el control de *Z. cucurbitae*.

Palabras Claves: *Zeugodacus*; *cucurbitae*; técnica de insecto estéril; señuelo marca-lanzamiento-recaptura

The true fruit flies (Diptera: Tephritidae) include over 4,000 species of which approximately 250 species are serious agricultural pests of fleshy fruits and vegetables (White & Elson-Harris 1992; Dhillon et al. 2005). Females typically oviposit in a variety of host plants, and the damage caused by the developing larvae may render the crops unmarketable (Sapkota et al. 2010). Even if control efforts successfully reduce damage, the associated costs may reduce profit margins substantially via increased overall production costs (Singh & Singh 1998). In addition, the risk of importing pest-ridden commodities often results in strict quarantine guidelines on exporting countries, thus hindering international trade (Jang et al. 2014). Despite improved management and regulatory practices, the high dispersal ability of fruit flies along with increased levels of global transport of people and goods have increased the invasion threat of pest tephritids (Qin et al. 2015).

Control of invasive fruit flies often adopts an Integrated Pest Management approach that includes detection and surveillance via trapping programs, application of synthetic insecticides and protein bait sprays, release of natural enemies, male annihilation technique, and the sterile insect technique (Klassen 2005; Dyck et al. 2006; Vargas et al. 2008, 2015). The latter tactic involves the mass production, sterilization, and release of the target pest to obtain sterile male × wild female matings, which yield infertile eggs and thus reduce population growth

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(Klassen 2005). The effectiveness of the sterile insect technique depends strongly on the survival, dispersal, and mating capabilities of the released sterile males (Calkins 1984), and accordingly sterile males are considered the "primary active agent" in the sterile insect technique (Franz & McInnis 1996). Consequently, the artificial selection that occurs in mass-rearing facilities may negatively impact various biological parameters of the released flies, including flight ability and dispersion, life span, and mating competitiveness. For example, Koyama et al. (1986) determined that a long-term lab stock of melon fly outcompeted wild males for females in small cages under high fly densities; however, in larger cages under lower density conditions the opposite occurred. Additionally, Koyama et al. (1986) observed that the long-term lab stock began mating significantly earlier in the d than their wild counterparts, which may decrease the likelihood of the sterile males mating with wild females, thus decreasing the effectiveness of the sterile insect technique program. In order to limit negative impacts from artificial selection, it is common to infuse lab stocks with wild individuals to increase the genetic diversity.

The sterile insect technique has been used successfully in eradication and control programs to control several economically important species, including the melon fly *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae). In a well-known instance, the Japanese government funded and successfully completed an intensive eradication effort to control *Z. cucurbitae*, in which the sterile insect technique was a key component (Koyama et al. 2004). More recently, results from field cage trials showed that sterile males from a pupal-color, genetic sexing strain of *Z. cucurbitae* (the so-called T1 strain) (McInnis et al. 2004) competed equally with wild males in obtaining copulations with wild females (McInnis et al. 2004; Shelly 2019). Consistent with that result, releases of this males-only strain were found to induce high levels of egg sterility in wild populations of *Z. cucurbitae* in Hawaii (McInnis et al. 2007).

The high mating competitiveness of sterile T1 males clearly indicates that this strain is a good candidate for the sterile insect technique, but performance measures for other biological parameters, particularly dispersal and survival abilities, are necessary to more completely evaluate the strain's potential value in the sterile insect technique for control of *Z. cucurbitae*. The primary objectives of the present study were to measure and compare (i) dispersal tendencies of T1 and wild males in an open field setting, and (ii) short-term survival rates of T1 and wild males in semi-natural, cage conditions. As measurement of dispersion relied on trapping using the male-specific attractant cue-lure (4-[p-acetoxyphenyl]-2-butanone) (Vargas et al. 2010), we initially compared attraction of T1 and wild males to cue-lure-baited traps in field cage and open field conditions. As described below, our experiments indicate that mature males of the T1 and wild strains did not differ noticeably in lure responsiveness or survival ability but did differ significantly in dispersal tendency.

Materials and Methods

INSECTS

All experiments involved comparisons between males from 2 strains of *Z. cucurbitae*. The white pupae, or so-called T1, strain is a pupal-color-based strain that was developed in 2001 (McInnis et al. 2004) and has since been reared continuously at USDA-ARS facilities in Hawaii, USA, most recently at the USDA-ARS Daniel K. Inouye Pacific Basin Agricultural Research Center in Hilo, Hawaii, USA. Screening and filtering were performed each generation to detect and eliminate recombinant individuals (in which sex linkage of the pupal color trait breaks down, causing mismatch between pupal color and sex), and thereby maintain the stability of the sexing mechanism (Fisher & Cáceres 2000). The wild strain was derived from approximately 1,500 adults reared from papaya (*Carica papaya* L.; Caricaceae) collected in commercial fields near Keaau, Hawaii. The wild flies used in the present study were 3 to 4 generations removed from the wild.

REARING, MARKING, AND MAINTENANCE

Both strains of *Z. cucurbitae* were reared following the protocol described in Vargas (1989) except that sliced papaya was provided as an ovipositional substrate for the wild strain, which was then placed on artificial diet. Papaya was placed on diet following oviposition, because it produces insects of more uniform quality as demonstrated in Manoukis et al. (2013). Following larval development, pupae (2 d before adult emergence) from the T1 strain and the wild colony were coated with different colors of fluorescent dye (arc yellow, neon yellow, horizon blue, or rocket red, from DayGlo Corporation, Cleveland, Ohio, USA) to distinguish males from the 2 strains in the mark-release-recapture experiments. Colors of fluorescent dye were alternated between treatments over successive trials (i.e., replicate 1: rocket red and neon yellow; replicate 2: arc yellow and horizon blue; replicate 3: rocket red and neon yellow; replicate 4: arc yellow and horizon blue, etc.). Upon emergence, the adult flies generally retain dye particles on the body that can be viewed with a dissecting microscope under ultraviolet (black) light. However, where external dye was not evident, the head was crushed with the blunt end of a dental instrument to examine the collapsed ptilinum, which picks up dye particles upon the fly's emergence from the puparium.

Emerged, pre-release adults were held in PARC boxes (0.48 × 0.60 × 0.33 m) (Rubbermaid Commercial Products Inc., Huntersville, North Carolina, USA), which are opaque, plastic boxes that contain mesh screening on the sides and top for ventilation. Adult food was provided as a granular mixture of 3:1 (v/v) sugar: a circular cake (6 cm diam × 2 cm thick) of protein yeast hydrolysate was placed on the top screen through which the flies could feed. Additionally, an agar block (15 × 10 cm × 5 cm thick) was provided as a water source. Both food and water were replaced after 7 d. These holding boxes were kept under the same environmental conditions as the rearing colonies (i.e., 22.5 ± 1 °C, 55% ± 3% RH, and a 14:10 [L:D] photoperiod).

With one exception (the "dispersal" experiment, see below), only males were released in the experiments. In these instances, PARC boxes were chilled in a walk-in cold room (3–4 °C) for 30 min, and males were collected, counted, and then transferred to cubical screen cages (25 cm per side; 500 males per cage), which were then held under the same rearing conditions noted above. Chilling and male collection were conducted 24 h prior to release, and food and water were provided to the males in the screen holding cages. For all experiments, T1 and wild males were 20 to 24 d old and 28 to 32 d old at the time of release, respectively.

MALE ATTRACTION TO CUE-LURE

Two experiments were performed to compare lure responsiveness between T1 and wild males. The first experiment was conducted using 4 large field cages (each 15 × 6 × 3 m, L:W:H) and located on a gravel area outside the USDA-ARS facility in Hilo, Hawaii (105 masl). In each cage, a large plastic delta trap (Scentry Biologicals Inc., Billings, Montana, USA) baited with a fresh 2 g cue-lure plug (Scentry Biologicals Inc., Billings, Montana) was placed on each of 4 trees that marked the corners of a rectangle (13.1 × 5.0 m) with its center being the center of the cage. In the trap, the cue-lure plug and half of a Hercon vaportape

II insecticidal strip (2.5 × 5 cm containing 0.295 g a.i.; Hercon Environmental, Emigsville, Pennsylvania, USA) were placed in a perforated basket suspended over the sticky floor. The corner trees included 2 curry berry (*Murraya koenigii* [L.] Spreng; Rutaceae) and 2 Kaffir lime (*Citrus hystrix* DC; Rutaceae) trees, and 2 additional individuals on each species were placed haphazardly within the central rectangle. Plants were of uniform size, with a height of 1 to 2 m and ground canopy cover of 1 to 2 m^2.

For each replicate, 1,000 T1 and 1,000 wild males were released from 4 screen cages placed at the center of the cage (7 m from each trap) at 9:00 AM. After 72 h, captured flies were identified under ultraviolet light. Ten releases were performed during Jan to Mar 2018, with a minimum of 7 d between successive releases. Four different cages (separated by a minimum of 20 m) were used for this experiment, and on all release dates flies were released in 2 of the cages (i.e., 20 replicates were performed in total). The alternation of cages and dye colors between successive releases allowed at least 28 d between the re-use of a particular dye color in a given cage. In general, releases occurred under cloudy skies with temperatures ranging from 20 to 27 °C and precipitation ranging between 0 to 35 mm over the 72 h test interval.

The second experiment assessing male attraction to cue-lure was conducted in a macadamia nut orchard (*Macadamia integrifolia* Maiden & Betche; Protaceae) in Keaau, Hawaii (170 masl). For a given replicate, 1,000 T1 and 1,000 wild males were released from 4 screen cages, each containing 500 individuals of a given strain, from a central release point at 9:00 AM. One Jackson trap (Scentry Biologicals, Inc., Billings, Montana) was hung on each of eight 1.5 m plastic posts that were placed 20 m from a central release point at the 4 cardinal directions (N, S, E, W) and intermediates (due NE, NW, SE, SW). Each trap was baited with a cotton wick soaked in 5 mL cue-lure containing 5% dimethyl 1,2-dibromo-2,2-dichloroethylphosphate ('dibrom') placed in a perforated basket suspended over the sticky floor. Traps were removed after 24 h, and captured flies were counted under ultraviolet light to allow for strain identification. Ten releases were performed during Mar to Jun 2018 with a minimum of 7 d between successive releases. As cue-lure has low volatility and prolonged attractiveness (Vargas et al. 2009), the cotton wicks were used for 2 consecutive replicates before being replaced. In this experiment, it was assumed that captured flies of a given dye color were from the most recent release using that color. In general, releases occurred under cloudy skies with temperatures ranging from 17 to 26 °C and precipitation ranging between 0 to 34 mm over the 24 h test interval.

DISPERSAL

Dispersal of T1 and wild males was monitored in the same macadamia nut orchard mentioned above. Trees were of uniform size, with height of approximately 5 m and ground canopy cover of approximately 20 m^2. Tree rows were 8 m apart, and within a row trees were spaced at 5 m intervals (trunk-to-trunk). The entire orchard covers approximately 450 ha. Macadamia is neither a food source nor ovipositional site, thus eliminating these potential influences on the dispersal of released flies.

Both sexes of both T1 and wild strains were released in this experiment. The number of males released was estimated by recording the total volume of pupae placed in an individual PARC box (36 pupae per mL) and making quality control measurements (following internationally accepted procedures by FAO/IAEA/USDA 2014) of adult emergence, flight ability, and sex ratio for flies from the same production batch as the released individuals. For wild *Z. cucurbitae*, adult emergence and flight ability rates averaged 69% ± 5 and 66% ± 4, respectively, while for the T1 strain, the average adult emergence and flight ability rates were 91% ± 3 and 86% ± 4, respectively. Each production batch from each strain possessed a 1:1 sex ratio. Approximately 4 h after each release, PARC boxes were examined and remaining males were counted to determine mortality. On average, the mortality rate for wild males was 17.1% ± 1.3, while the corresponding value for T1 males was 8.9% ± 0.3.

Flies were released from a central release point (2 PARC boxes per strain) at 9:00 AM. A total of 64 Jackson traps were uniformly spaced throughout a 1,050 m × 1,040 m grid with trap distances ranging from 150 to 739 m from the central release point. Traps were removed 72 h after release, and captured flies were counted under ultraviolet light to allow for strain identification. Seven releases were performed during Nov to Dec 2018 with a minimum of 7 d between successive releases. As above, we assumed that captured flies of a given dye color were from the most recent release using that color. Based on the volume of pupae placed in the PARC boxes and the biological performance parameters, the numbers of T1 males released varied from 2,399 to 2,779 per replicate, and corresponding estimates for wild males ranged between 1,332 and 1,664 individuals per replicate. In general, releases occurred under partly cloudy skies with temperatures ranging from 19 to 23 °C and precipitation ranging between 0 to 93 mm over the 72 h test interval.

MALE SURVIVORSHIP

Fifty newly emerged T1 or wild males were placed in individual screened cages (1 × 1 × 1 m) held on a covered porch outside the laboratory in Hilo, Hawaii. Food and water were provided as a mixture of water, sugar, yeast hydrolysate, honey, and agar (100:30:10:7.5:1 w/w) as a circular slab (9 cm diam × 1.5 cm thick) in a Petri dish covered with a screen through which the flies could feed. Dead flies were removed and counted every 3 d for 42 d. Six cages were observed for each strain during May to Jul 2019. During this interval, temperature ranged from 18 to 31 °C and humidity was 65 to 100%.

STATISTICS

In the experiments measuring response to cue-lure and survivorship, T1 and wild males were compared using a paired *t*-test (2-tailed); ln transformed raw data were normally distributed (based on the Shapiro-Wilk test). In the dispersion experiment, release numbers differed between T1 and wild males, and consequently trap counts were transformed to proportions (captures per estimated release numbers). The proportional data were pooled over 3 distance categories, 150, 260 to 397, and 450 to 739 m from the release point, respectively, and then analyzed using a non-parametric variation of a 2-way ANOVA. Following Conover and Iman (1981), data were ranked over all 7 releases and the 3 distance categories, and these ranks were then subject to a 2-way ANOVA with male strain and distance as the main effects. Tukey's multiple comparison test was used to investigate pair wise differences. Computations were performed using JMP, vers. 12 (SAS Institute Inc., Cary, North Carolina, USA).

Results

MALE ATTRACTION TO CUE-LURE

No differences in recapture rates were detected between T1 and wild *Z. cucurbitae* males in either the large field cage or open field circular plot experiments. In the field cage trials, the mean numbers of males captured in the cue-lure-baited traps per replicate were 226.4 ± 67.9 and 232.1 ± 58.3 for T1 and wild males, respectively (t = 0.285; df = 19; P = 0.28). Similarly, in the field releases, the mean numbers of

males captured per replicate were 106.3 ± 20.5 and 102.1 ± 14.2 for T1 and wild males, respectively (t = 0.46; df = 9; P = 0.65).

DISPERSION

Based on the ANOVA on ranked values, both male strain ($F_{1, 36}$ = 44.6; P < 0.001) and distance from the release point ($F_{2, 36}$ = 174.7; P < 0.001) had significant effects on recaptures (Fig. 1). The interaction term was not significant ($F_{2, 36}$ = 2.4; P = 0.10). Tukey's test revealed that wild males were captured in significantly higher proportions than T1 males at all 3 distance categories (P < 0.05 in all cases). Additionally, recapture proportions were greater at 150 m than 260 to 397 m, and at 260 to 397 m than 450 to 739 m for both the wild and T1 strains (P < 0.001 in all cases). Overall the proportion of recaptures were 16% and 28% for T1 and wild males, respectively.

MALE SURVIVORSHIP

No differences in survivorship rates were observed between T1 and wild *Z. cucurbitae* males housed in screen cages exposed to outdoors conditions. At the conclusion of the survivorship trials the mean numbers of males per replicate that survived the 42 d experimental period were 44.6 ± 2.3 and 45.5 ± 2.6 for T1 and wild males, respectively (t = 0.57; df = 5; P = 0.58).

Comparisons between the T1 and wild strains with respect to cue-lure attraction, survivorship, and dispersal are summarized in Table 1.

Discussion

Release-recapture data under cage and field conditions revealed no significant difference in attraction to cue-lure-baited traps between genetic sexing strain T1 and wild *Z. cucurbitae* males. Similar findings were reported previously for bisexual laboratory and wild strains of this species (Wong et al. 1991; Manoukis & Gayle 2015), but the present data represent the first published comparison of genetic sexing strains and wild strains for *Z. cucurbitae*. Few comparable studies exist for other economically important tephritid species. In the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), results have been inconsistent: Shelly and Edu (2009) reported that wild males were more responsive to the male lure trimedlure than laboratory males; Wong et al. (1982) found the opposite trend; and Barry et al. (2003) reported no difference in lure attraction between mass-reared and wild males. In the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), laboratory and wild males were found to respond similarly to monofluoro analogues of the male lure methyl eugenol (Liquido et al. 1998). Additional studies of this type are clearly needed, because data collected on mass-reared flies often are used to make inferences about wild flies, a practice that may or may not be reliable (Peck & McQuate 2004; Weldon et al. 2014).

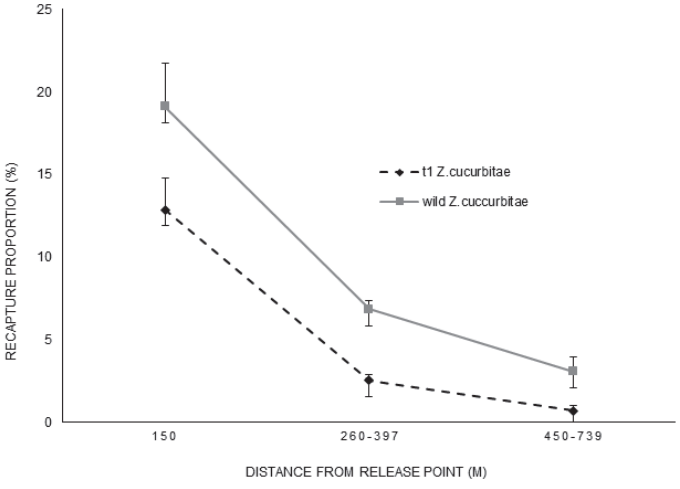


Fig. 1. Proportions of marked and released T1 and wild *Zeugodacus cucurbitae* males captured in cue-lure-baited traps at 3 distances from a central release point in a macadamia orchard. Points represent mean values (± 1 SE) for 7 replicates based on the ANOVA on ranked values; both male strain ($F_{1, 36}$ = 44.6; P < 0.001) and distance from the release point ($F_{2, 36}$ = 174.7; P < 0.001) had significant effects on recaptures. Tukey's test revealed that wild males were captured in significantly higher proportions than T1 males at all 3 distance categories (P < 0.05 in all cases). Additionally, recapture proportions were greater at 150 m than at 260 to 397 m, and at 260 to 397 m than at 450 to 739 m for both the wild and T1 strains (P < 0.001 in all cases).

Based on caged populations subject to ambient conditions of light, temperature, and humidity, we found no difference in longevity between T1 and wild *Z. cucurbitae* over the 42-d monitoring period. At the conclusion of the 42-d experiment survival proportions were 89 ± 2.3 and 91 ± 2.6% for T1 and wild *Z. cucurbitae* males, respectively. Whether this result holds for field estimates of survival rates, of course, remains unknown. Based on trap captures monitored at 2 to 3 d intervals over several wk on Okinawa, Nakamori and Soemori (1981) concluded that daily survival rates were greater for wild males than for mass-reared males from a bisexual strain. However, inter-strain differences in survivorship increased with time, and data from the first sampling interval (i.e., 2–3 d post-release) showed small or no differences in recapture rates of wild and laboratory males. Thus, because recaptures in our study were scored 72 h after release, we assume that survival rates prior to trap collection did not differ greatly between T1 and wild males.

Given the similarity in lure attraction and survivorship of the 2 strains, the results of the release-recapture field study indicate that wild males showed greater dispersion than their T1 counterparts. In the present study, recapture proportions for wild males were significantly greater at each of 3 measured distance categories (150, 260–397, and 450–739 m). For example, recapture rates for wild males at each of the 3 distance classes were 19.12% ± 7.0, 6.84% ± 1.3, and 3.1% ± 2.5, respectively, whereas the T1 *Z. cucurbitae* males were recaptured at significantly lower rates: 12.88% ± 7.1, 2.53% ± 0.3, and 0.69% ± 0.3, respectively. These data are consistent with Nakamori and Soemori (1981), who found that several d after release of *Z. cucurbitae*, most laboratory-reared males were captured within 50 m of the release point, whereas wild males were often captured 100 to 200 m from the release point. Greater movement of wild males relative to laboratory males also was reported for the olive fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) (Fletcher & Economopoulos 1976). In contrast, laboratory-reared and wild males of *Anastrepha ludens* (Loew) and *A. obliqua* (Macquart) (both Diptera: Tephritidae) displayed similar levels of movement (Hernández et al. 2007). Although trapping area as well as trap density vary among dispersal studies of *Z. cucurbitae*, the overall proportion of recaptures reported here (16% and 28% for T1 and wild males, respectively) was similar to that reported by Hamada (1980; 12%–15% for mass-reared males) and Peck et al. (2005; 8%–53% for laboratory-reared males), with all these studies employing cue-lure-baited traps. Nakamori and Soemori (1981) sampled a much larger area (circle with 1 km radius) with a relatively small number of cue-lure-baited traps, and accordingly reported recapture rates of only 1% to 9% for mass-reared males and 2% to 15% for wild males.

Two key points should be considered when interpreting the dispersal data presented here. First, owing to a lack of a suitable radiation source on Hawaii Island, the T1 males were "untreated" or "normal,"

Table 1. Summary of comparisons between T1 and wild strains of *Z. cucurbitae*. For cue-lure attraction, values represent mean numbers (± 1 SE) of males captured at a cue-lure source with 1,000 males released per strain per replicate for both experiments. For survivorship, values represent mean numbers (± 1 SE) of males that survived 6 wk in cages with 50 males placed per cage per strain. For dispersal, values represent mean proportions (± 1 SE) of males that were captured in cue-lure-baited traps at 3 distance categories from a central release point with at least 1,332 males released per strain per replicate. See text for additional details.

Parameter	T1	Wild	*P*	Comparison
		Attraction to cue-lure (no.)		
Field cage	226.4 (67.9)	232.1 (58.3)	0.28	T1 = Wild
Field plot	106.3 (20.5)	102.1 (14.2)	0.65	T1 = Wild
		Survivorship (no.)		
	44.6 (2.3)	45.5 (2.6)	0.58	T1 = Wild
		Dispersal (%)		
150 m	12.88 (7.1)	19.12 (7.0)	P < 0.001	Wild > T1
260–397 m	2.53 (0.3)	6.84 (1.3)	P < 0.001	Wild > T1
450–739 m	0.69 (0.3)	3.1 (2.5)	P < 0.001	Wild > T1

i.e., not irradiated. Thus, we do not know whether the results obtained necessarily apply to sterile males. Surprisingly, given the importance of dispersal of released males in the sterile insect technique programs, few studies have compared movement of mass-reared, sterile males to either mass-reared, non-irradiated males or wild males (Weldon et al. 2014). Regarding *Z. cucurbitae*, Hamada (1980) found that mass-reared, irradiated males (100 Gy) were recaptured at lower rates than mass-reared, non-irradiated males, but that travel distances did not differ markedly between irradiated and fertile males. As noted above, Nakamori and Soemori (1981) reported that wild males dispersed greater distances than mass-reared, non-irradiated males. Note that these studies (along with the present study) do not allow definitive conclusions regarding the possible effects of mass-rearing or sterilization on dispersal, because they did not include both irradiated and non-irradiated mass-reared males. Interestingly, a literature survey of the Tephritidae revealed no overall pattern regarding effects of domestication or sterilization on dispersal (Weldon et al. 2014). For example, in the Queensland fruit fly, *Bactrocrea tryoni* (Froggatt) (Diptera: Tephritidae), dispersal distance was similar among mass-reared males (both irradiated and non-irradiated) and wild males (Weldon & Meats 2010), and, in two *Anastrepha* species, mass-reared, sterile males and wild males dispersed similar distances after release (Hernández et al. 2007).

Second, dispersion was investigated in a habitat that was not particularly favorable for *Z. cucurbitae* owing to the absence of host plants and associated roosting sites (i.e., preferred "refuge" or resting sites adjacent to areas containing host plants) (Nishida & Bess 1957; Stark 1995). Several studies (Hamada 1980; Koyama et al. 1982; Peck et al. 2005) indicate that dispersal tendency of *Z. cucurbitae* is inversely related to habitat suitability. This trend appears to characterize other tephritid species as well, e.g., *B. dorsalis* (Iwahashi 1972), *B. oleae* (Fletcher & Economopoulos 1976; Fletcher & Kapatos 1981), and *B. tryoni* (Sonleitner & Bateman 1963). Thus, in the present study, the recapture proportions in the more distant traps would likely exceed those obtained for releases in areas with abundant host plants. Independent of travel distances observed, it should be noted that among cue-lure-baited traps deployed in the field, several studies on *Z. cucurbitae* (Hamada 1980; Koyama et al. 1982; §Iwaizumi & Shiga 1989) have documented a positive correlation between the numbers of released, mass-reared males and naturally occurring wild males. Thus, released mass-reared males of *Z. cucurbitae* tend to aggregate in the same areas having a high abundance of wild males, a finding advantageous for the success of the sterile insect technique programs.

The data presented here contribute to the overall assessment of the T1 pupal sexing strain as a candidate for the sterile insect technique programs for control of *Z. cucurbitae*. Earlier studies present ambiguous results regarding this strain's suitability. On one hand, measurement of various rearing parameters (i.e., egg production and hatch, pupal production, etc.) showed that for a given quantity of eggs, the expected yield of adult flies was approximately 50% lower for the T1 strain compared to a bisexual strain (Fezza et al. 2018). Thus, roughly twice as many resources would be required to rear the genetic sexing strain relative to the bisexual strain. On the other hand, field cage trials showed that sterile T1 males competed equally with wild males for copulations with wild females (Shelly 2019), a key finding as high mating ability of released males is critical to the success of any sterile insect technique project (Calkins 1984). In *C. capitata*, where sterile mass-reared males are inferior to wild males in sexual competition (Shelly & McInnis 2016), male-only releases were found to induce much higher levels of egg sterility than bisexual releases (Rendon et al. 2004). Presumably then, the release of highly competitive, sterile T1 males only would greatly improve the efficacy of the sterile insect technique and result in relatively high levels of egg sterility. The present study also shows that, at least in semi-natural conditions, T1 males have survival ability comparable to wild males. The consequence of the low dispersal of T1 males may vary with the size of the area included in a sterile insect technique program. If the invasive population is detected early and is confined to one or a few localities, then the movement of T1 males may be sufficient to locate mating and roosting sites of wild flies. However, infestations occurring over very large areas may require a relatively large number of release transects or release points to insure adequate coverage by the sterile males.

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