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Source: Florida Entomologist, 96(3) : 839-849

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.096.0318

ABILITY OF GENETIC SEXING STRAIN MALE MELON FLIES (DIPTERA: TEPHRITIDAE) TO SUPPRESS WILD FEMALE REMATING: IMPLICATIONS FOR SIT

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ABSTRACT

For successful application of the sterile insect technique (SIT), wild female insects should not be more receptive to remating after mating with a mass-reared sterile male than after to mating with a wild fertile male. The remating frequencies of melon fly *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) females were assessed in field cages with male melon flies from: (1) a male-only genetic sexing strain (GSS) originating from Hawaii, (2) a bisexual (male and female) laboratory strain originating from Mauritius, and (3) a wild colony (less than 5 generations in culture) also from Mauritius. One objective of this study was to assess the ability of GSS males to suppress the remating of females of different strains as compared to the ability of males of bisexual strains to do so. A second objective was to assess the effect of mass-rearing and irradiation on the ability of GSS males to suppress female remating. The males of the GSS achieved significantly fewer matings with female flies from the laboratory adapted and wild strains during the first mating than males of these bisexual strains. However, GSS males were equally able to diminish the females' remating frequency as laboratory and wild males. Remating frequencies of GSS females were significantly higher than those of females of the bisexual strains. Our results, however, indicate that laboratory rearing had no effect on the remating frequency of melon fly females. Thus the higher remating frequency of GSS females seemed to be a strain specific characteristic. Furthermore, irradiation of male melon fly pupae with 70 Gy had no effect on female remating frequencies, and the abilities of irradiated GSS and wild males to suppress wild female remating were similar. These results are discussed in the context of the feasibility of incorporating the use of irradiated GSS males as the SIT component of area-wide pest management programs against *B. cucurbitae*.

Key Words: *Bactrocera cucurbitae*, remating behavior, mating compatibility, female receptivity, genetic sexing, sex separation

RESUMEN

Para la aplicación exitosa de la técnica del insecto estéril (TIE), las hembras salvajes no deberían ser más receptivas a copular después de aparearse inicialmente con un macho estéril criado masivamente en condiciones de laboratorio al igual que después de copular con un macho fértil salvaje. Se evaluó la frecuencia de copula de las hembras de la mosca del melón *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) en jaulas de campo con machos de la mosca del melón utilizando: (1) una cepa en la cual sus características genéticas permiten separar y liberar solamente machos (GSS por sus siglas en inglés) procedente de Hawái, (2) una cepa bisexual (machos y hembras) de laboratorio procedente de Mauricio y (3) una colonia silvestre (criada en el laboratorio por menos de 5 generaciones) también procedente de Mauricio. Un de los objetivos de este estudio fue evaluar y comparar la capacidad de suprimir la re-copula de las hembras salvajes después de que estas copulan inicialmente con los machos GSS, con los machos de la cepas bisexuales o con los machos salvajes. Un segundo objetivo fue evaluar el efecto de la cría en masa y la irradiación sobre la capacidad de los machos GSS en suprimir la re-copula de las hembras salvajes. Los machos de la GSS lograron significativamente menos copulas con hembras de la cepa de laboratorio y las hembras silvestres durante el primer apareamiento que los machos de la cepa bisexual. Sin embargo, los machos de GSS fueron igualmente capaces de disminuir la frecuencia de copula de las hembras salvajes tanto como los machos de la cepa bisexual de laboratorio y los machos sal-

vajes. La frecuencia de apareamiento de las hembras de GSS fue significativamente mayor que la de las hembras de las cepas bisexuales. Nuestros resultados, indican que la cría en masa en el laboratorio no tuvo efecto sobre la frecuencia de re-copula de las hembras de la mosca del melón. La frecuencia más alta de apareamiento de las hembras de GSS aparentemente es una característica específica de la cepa. Además, no se observó ningún efecto de la irradiación después de que las pupas fueran irradiadas con 70 Gy. Los machos irradiados GSS y los machos salvajes suprimieron similarmente la re-copula de las hembras salvajes. Se comenta sobre estos resultados en el contexto de la factibilidad de incorporar el uso de machos irradiados de GSS como componente de TIE en los programas de control en áreas extensas contra *B. cucurbitae*.

Palabras Clave: *Bactrocera cucurbitae*, comportamiento apareamiento, compatibilidad de apareamiento, receptividad femenina, sexos genéticos, separación de sexos

The melon fly *Bactrocera cucurbitae* Coquillett (Diptera: Tephriditae) is an economically important pest of fruits and vegetables (White & Elson-Harris 1992). It causes severe direct losses by damaging cucurbit fruits and vegetables but has also indirect economic implications in view of its quarantine status as the fly's presence seriously interferes with the international marketing of these agricultural commodities. Relying on the indiscriminate use of traditional broad spectrum residual chemicals for the control of tephritid pests (Roessler 1989) entails increased environmental concerns and difficulties to access export markets such as the European Union that have very stringent import conditions in relation to insecticide residues.

The sterile insect technique (SIT) (Dyck et al. 2005), applied as a component of an area-wide integrated pest management (AW-IPM) approach (Klassen & Curtis 2005; Hendrichs et al. 2007), is a well-established environment-friendly control tactic that has been successfully used against various dipteran (fruit flies, screwworms, tsetse flies), lepidopteran and coleopteran pests (Vreysen et al. 2000; Wyss 2000; Kohama et al. 2003; Ledford 2010). It has also been used for melon fly suppression (Vargas et al. 2004; Jang et al. 2008) and eradication (Kakinohana 1994). The SIT is based on the mating of released sterile males with wild virgin females that then will produce no viable offspring (Knipling 1955). The technique requires the rearing of the target insect in large numbers in specialized factories, the sexual sterilization with ionizing radiation, and their sequential release over the target area in numbers large enough to minimize matings between males and females of their wild counterparts.

The eradication of the melon fly from the Okinawa archipelago in Japan is undoubtedly the best known AW-IPM programme with an SIT component against this pest (Kakinohana 1994). Although both sterile male and female flies were released together (bisexual strain used), the melon fly was successfully eradicated in 1993. Since then, several developments have increased the cost-effectiveness of the SIT application against different fruit fly species, especially for the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). Two advances were of particular significance: (1) the development of genetic sexing strains (GSS) allowing the release of male flies only, and (2) major progress with the industrial production and performance enhancement of sterile male flies on a large scale making the SIT more cost effective (Hendrichs et al. 2002; Pereira et al. 2011).

In most cases the release of sterile female flies does not contribute to controlling the pest. Their production and release are therefore unnecessary from a biological and technical point of view. Using classical genetic approaches, GSS have been developed that enabled the separation of male from female fruit flies at some point during their development in the production process (Robinson et al. 1999). The removal of female flies from the production line has important economic implications in terms of reduced rearing, transport and release costs (Cáceres et al. 2002). In addition, releasing only sterile male fruit flies increases the effectiveness of the SIT significantly as sterile males appear to disperse farther in the absence of sterile females, and do not waste their limited sperm on sterile females but focus on competing with wild males for wild females (McInnis et al. 1994; Hendrichs et al. 1995). Furthermore, damage in some fruit varieties caused by the 'stings' of sterile females is avoided, and a high sex-ratio of sterile males to wild females can be maintained (McInnis, Tam, Grace & Miyashita 1994; Rendón et al. 2000).

In view of all the above mentioned advantages of male-only releases, efforts were undertaken to develop genetic sexing strains of *B. cucurbitae*, which have so far resulted in a strain based on a sex-linked difference in pupal color (McInnis et al. 2004). The incorporation of this GSS into SIT programs against *B. cucurbitae* would permit the automatic separation of male (wild type brown pupae) from female flies (mutant white pupae) in a pupae separator (McInnis et al. 2004). Recent studies have indicated that *B. cucurbitae* males of this GSS, which originated from a Hawaii population, are sexually competitive with male flies from populations of 2 other geographical regions,

Mauritius and Seychelles (Sookar et al. 2010) and these data support the potential use of this GSS in sterile insect release programs against *B. cucurbitae* in different countries.

Adequate mating competitiveness of mass-reared sterile male flies is a basic requirement for the successful application of the SIT. Sterile male flies should not only be competitive with wild males when competing in leks for mating with wild females, they should also be capable of transferring during mating an adequate complement of male ejaculate. In addition, wild females that mated with mass-reared males should ideally not be more receptive to remating than females that mated with fertile wild males (Hendrichs et al. 2002).

Polyandry in female *B. cucurbitae* is a common phenomenon, and remating frequencies are influenced by factors such as duration of the first mating (Yamagishi & Tsubaki 1990; Kuba & Itô 1993), the strain (I. Haq, unpublished data), and the number of generations that a population has been cultured in the laboratory or mass-rearing facility (Kuba & Itô 1993; Nitzan et al. 1993; Vera et al. 2003). On the other hand, factors such as the quantity of sperm transferred (Yamagishi & Tsubaki 1990; Kuba & Itô 1993) and those that enhance the sexual performance of the male flies, such as the topical application of the juvenile hormone analog, methoprene, and protein supplements to the diet (Haq et al. 2010), have no effect on female remating frequencies (I. Haq, unpublished data). Haq (unpublished data) noted a significantly higher remating frequency in *B. cucurbitae* females of the GSS than in melon fly females of the much studied bisexual strain used in the SIT programme in Okinawa, Japan. It was, however, not clear whether this higher remating frequency was due to factors related to mass-rearing, to an intrinsic characteristic of the GSS females, or to the inability of the GSS males to suppress or diminish female remating. The latter inability could negatively affect the efficiency of a release programme that uses males from this GSS, and it was therefore deemed important to further investigate this phenomenon. Therefore the objective of the research presented in this paper was to assess the ability of GSS males to suppress or diminish remating in wild type *B. cucurbitae* females in comparison to that achieved by wild or laboratory-adapted males of bisexual strains. The null hypothesis was that remating frequencies of the female flies would be similar in crosses with the 3 types of males.

Materials and Methods

Strain and Rearing

The following strains were used in the experiments: (1) the genetic sexing strain (GSS) developed by United States Department of Agriculture-Agricultural Research Service in Hawaii that had been maintained in the laboratory for ca. 60 generations (hereafter called Lab-GSS) (McInnis et al. 2004), (2) a laboratory adapted bisexual strain (Lab-Bisex) originating from Mauritius and maintained for ca. 45 generations in the laboratory, and (3) a wild strain (Wild-Strain) also originating from Mauritius and in culture for less than 5 generations (F_1 in experiment 1 and F_4 in experiment 3). The colonies were maintained at the FAO/IAEA Insect Pest Control Laboratory, Seibersdorf, Austria, on a wheat-based modified standard Seibersdorf larval diet (Hooper 1987). Following emergence, the flies were sexed and provided with a protein-rich diet in a 3: 1 ratio of sugar: hydrolyzed yeast and water ad libitum. The flies were maintained in an insectary at 14:10 h L:D, 24 ± 1 °C and 60 ± 5% RH.

Field Cages

All experiments were conducted in screened field cages (4 m^2 base × 1.8 m high) with a potted *Citrus sinensis* (Osbeck) tree, a non-host tree (height 1.7 m with a canopy of about 1.5 m diam.). Although a tree is required to study the mating behavior of melon fly in a semi-natural field cage setting, it does not have to be a host plant as melon fly mating occurs on diverse trees at the edges of melon fields (Iwahashi & Majima 1986). The field cages were located in a temperature-controlled glass greenhouse with natural light conditions and 25 ± 2 °C and 60 ± 5% RH during the experiments.

Experiment 1. Evaluation of Remating Frequencies of Lab-GSS and Lab-Bisex Females Mated with Lab-GSS and Lab-Bisex Males

Sexually mature flies (16 ± 2 days of age) were used in this experiment. Fifty Lab-GSS or 50 Lab-Bisex females were exposed in separate field cages to an equal number of virgin Lab-GSS or Lab-Bisex males for a total of 50 pairs per cage. Each of the 4 combinations (Lab-GSS ♂ × Lab-GSS ♀, Lab-Bisex ♂ × Lab-GSS ♀, Lab-GSS ♂ × Lab-Bisex ♀, and Lab-Bisex ♂ × Lab-Bisex ♀) of matings was carried out simultaneously at 1 replicate per day for a total of 4 replicates. Male-female combinations were rotated among field cages during replications of the experiment to compensate for any possible effect of environmental conditions of a particular field cage on mating success. Males were released 90 min before sunset followed by females 15 min thereafter. As soon as mating pairs were formed, time was recorded and the mating couples were collected separately in plastic vials and allowed to complete their mating. Coupling was observed until pair-formation ceased and essentially complete darkness at 90

min after sunset. The next morning, females were removed from vials and provided with sugar and water for 1 day. Forty-eight h after the first mating, 30 (the lowest number in one of the treatments) of the previously mated females were exposed again to equal numbers of virgin males of the same type as in the first mating and using the same field cage methodology. Kuba & Soemori (1988) had reported that *B. cucurbitae* females (bisexual strain from Okinawa, Japan) that were in copula less than 3 h remated within 2 days after their first mating, but females that were in copula for more than 10 h remated 12 days after their first mating. However, Haq (unpublished) found that duration of copulation had no effect the remating frequency of the genetic sexing strain (GSS) of *B. cucurbitae* females, because the GSS females (>80%) remated 48 h after the first mating. Therefore, the remating frequency of Lab-GSS females vs. that Lab-Bisex females was evaluated 48 h after the first mating. Remating frequencies of females after more than 48 h were not evaluated because for effective SIT application, independently of the remating frequency of wild females, matings by mass-reared sterile males should reduce the remating frequency of wild females to the same degree as occurs following matings wild male and wild females (Whitten & Mahon 2005). The males provided for the second mating were from the same batch of pupae as those involved in the first mating.

Experiment 2. Evaluation of Remating Frequencies of Lab-GSS and Wild-Strain Females Mated with Lab-GSS and Wild-Strain males

A similar methodology was used as in the first experiment except that (1) males and females (age 2 ± 2 days) of the Wild-Strain were used instead of the Lab-Bisex strain, and (2) 25 males and previously mated females of each strain were used in the second mating.

Experiment 3. Evaluation of the Effect of Irradiation of Male Flies on Remating Frequencies of Wild-Strain Females

A batch of wild fly pupae was divided into 2 groups of which one was exposed to 70 Gy of gamma rays in a ^{60}Co Gammacell 220 on the 9th day after pupation (2 days before emergence). Adult female flies that emerged from irradiated pupae were discarded, and only the wild females emerging from untreated pupae were used in the experiment. For the Lab-GSS flies, only brown male pupae were irradiated at the same dose and used for the experiment. Fifty virgin Wild-Strain females were exposed simultaneously in separate field cages to 50 Lab-GSS irradiated males or 50 Wild-Strain irradiated males. The experiment was replicated 4 times and 2 replicates were carried out each day. A similar protocol and methodology for the first and second mating was used as in experiment 2 except that the wild flies were 15-16 days of age at the time of the first mating. The flies used in the entire experiment were from the same batches of pupae.

Data Analysis

Data on male mating success were analyzed by analysis of variance (ANOVA) if the basic assumption of normality of data distribution was met, and complementary pairwise comparisons were carried out by Tukey's HSD test. As the effect of replicates was non-significant, it was removed from the model to increase the power of analysis. The male's ability to suppress female remating was analyzed by binary regression. Mating latency, defined as the time elapsed between the release of female flies in the field cage until initiation of a given mating, was also calculated. The latency data for each individual couple were subjected to logarithmic transformation and one-way ANOVA were performed using a General Linear Model followed by pair-wise comparisons Scheffe's Test (suitable for unequal sample size). The significance level used in tests was 95% (α = 0.05). Data were analyzed using Statistica software (StatSoft 2000).

Results

Experiment 1. Evaluation of Remating Frequencies of Lab-GSS and Lab-Bisex Females Mated with Lab-GSS and Lab-Bisex Males

While Lab-GSS females were equally likely (> 82%) to mate with Lab-GSS males and Lab-Bisex males, Lab-Bisex females were significantly less likely to mate with Lab-GSS males than Lab-Bisex males in the first mating (Fig. 1). The remating frequency of Lab-GSS females remained significantly higher (83%) than that of Lab-Bisex females (<53%) in the second mating (Fig. 1). However, the first mating with Lab-GSS males and Lab-Bisex males had a similar effect on remating frequency of Lab-GSS ($F_{1,238} = 0$, $P = 1$) and Lab-Bisex female flies ($F_{1,238} = 2.4$, $P = 0.2$).

Experiment 2. Evaluation of Remating Frequencies of Lab-GSS and Wild-Strain Females Mating with Lab-GSS and Wild-Strain Males

Overall mating success of Lab-GSS and Wild-Strain males was significantly different with Lab-GSS than with Wild-Strain females ($F_{3,12} = 26.95$, $P < 0.01$). In the first mating, Lab-GSS females were equally mating with a Wild-Strain male as with a Lab-GSS male, whereas Wild-Strain females were less likely to mate as compared to Lab-GSS females and had a significantly lower mating frequency with Lab-GSS males than with Wild-Strain males (Fig. 2).

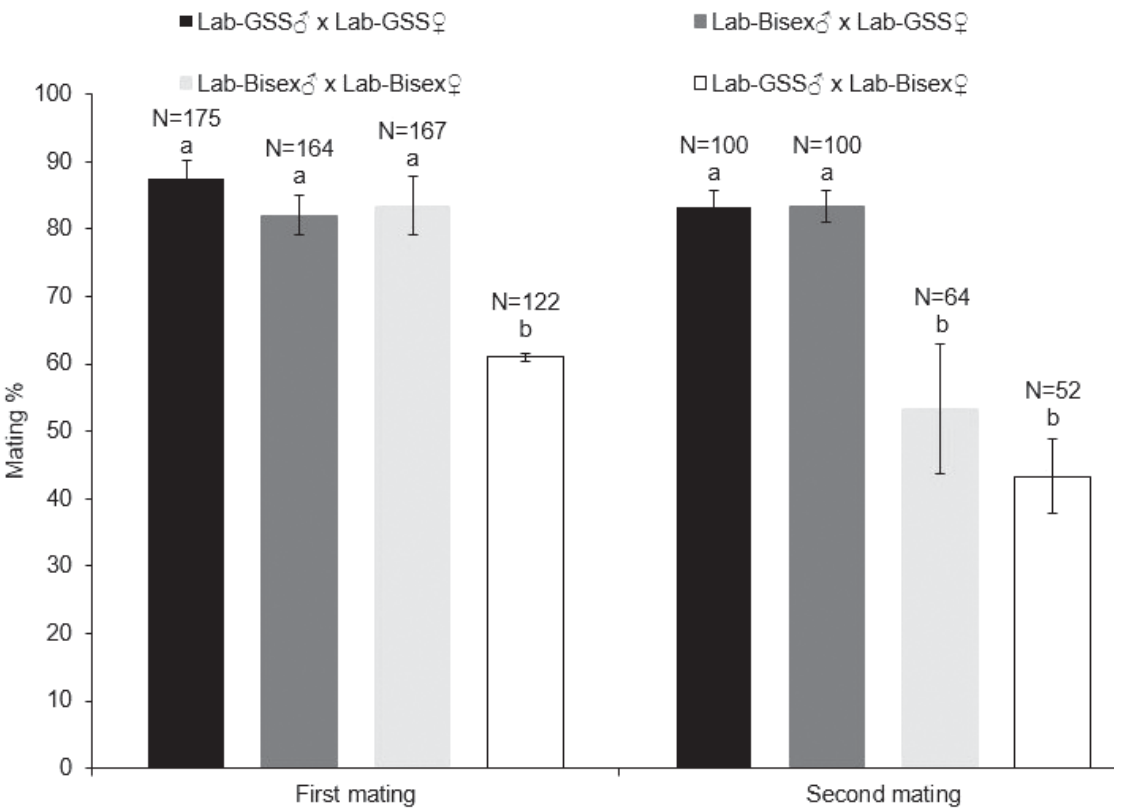

Fig 1. The percentage (mean ± S.E.) of *Bactrocera cucurbitae* females mating during a 1st and 2nd mating opportunity. The females, both Lab-GSS and Lab-Bisex,were exposed in separate field cages either to virgin males of a genetic sexing strain (F_{60} in laboratory rearing; Lab-GSS) or males of a bisexual laboratory strain (F_{45} in laboratory rearing; Lab-Bisex) originating from Mauritius. Females, who had successfully secured a mate during the 1st mating, were offered a 2nd mating opportunity 48h later by exposure to the same type of virgin males at a 1:1 sex ratio. The comparisons of number of matings (N) for 1st and 2nd mating were analyzed separately, with different letters indicating significant difference from each other (Tukey's test, $P < 0.05$).

In the second mating, remating rate of Lab-GSS females remained high, with no significant differences in their matings with Wild-Strin males and Lab-GSS males ($F_{1,198} = 0.1$, $P = 0.74$). Remating frequencies of Wild-Strain females were significantly lower as compared to the Lab-GSS females ($F_{1,398} = 31.1$, $P<0.01$), but Wild-Strain and Lab-GSS males were accepted at the same rate ($F_{1,198} = 0.31$, $P = 0.57$).

Experiment 3. Evaluation of the Effect of Irradiation of Male Flies on Wild-Strain Female Remating Frequencies

Irradiated Lab-GSS males had a lower mating success with Wild-Strain females in the first mating than irradiated Wild-Strain males ($F_{1,6} = 12.2$, $P = 0.01$). Remating frequencies of Wild-Strain females in the second mating was reduced to 29% with Lab-GSS males (as compared to 65% in the first mating) and to 43% with Wild-Strain males (as compared to 77% in the first mating), but the differences between the 2 male types during the second mating were not significantly different ($F_{1,6} = 4.5$, $P = 0.07$) (Fig. 3).

Mating Latency

The mating latency data are presented in Table 1. In the first experiment the mating latencies between Lab-GSS flies and Lab-Bisex flies were significantly different ($F_{3,630} = 46.8$, $P<0.01$) during the first and second mating ($F_{3,315} = 26.0$, $P < 0.01$). In the first mating, the mating latency between Lab-GSS males and Lab-GSS females was very short (30 ± 2.6 min), while it was 3 times as long for Lab-Bisex males and females (95 ± 3.6 min). The mating latencies of the other crosses (Lab-GSS ♂ × Lab-Bisex ♀ and Lab-Bisex ♂ × Lab-GSS ♀) were intermediate. Similar observations were made for the second mating.

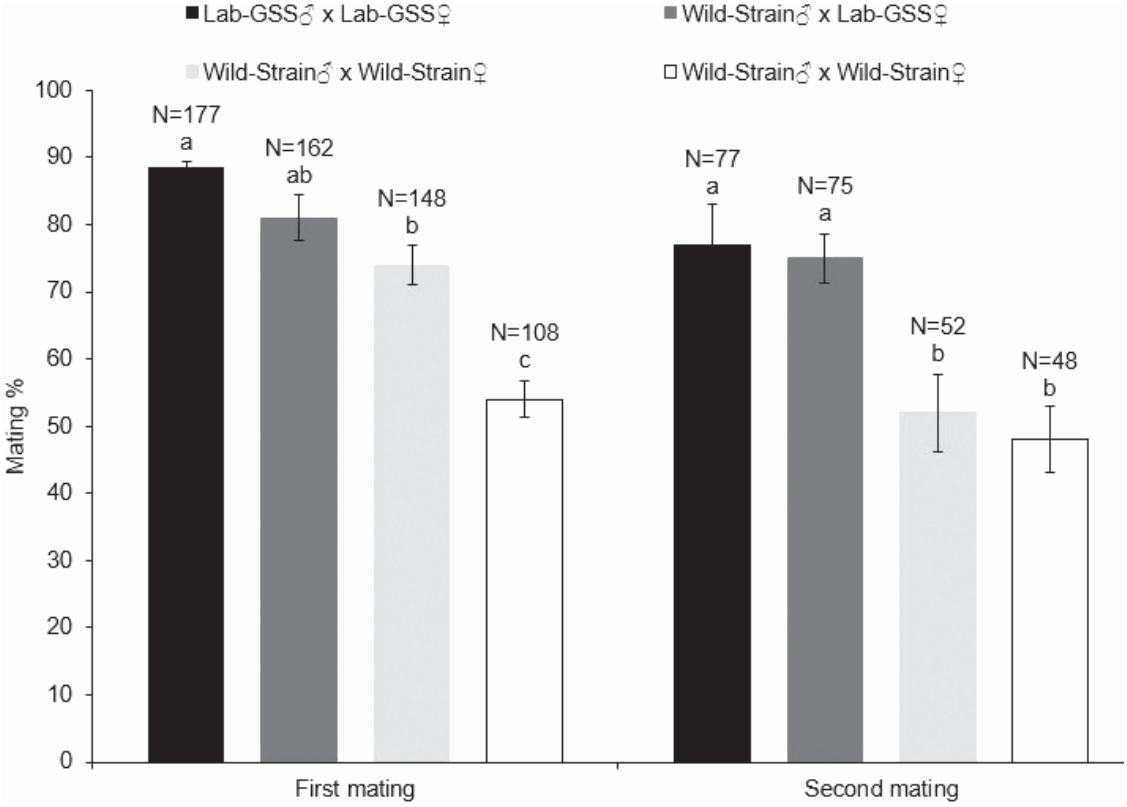

Fig 2. The percentage (mean ± S.E.) of *Bactrocera cucurbitae* females mating during a 1st and 2nd mating opportunity. The females, both Lab-GSS and Wild-Strain, were exposed in separate field cages either to virgin males of a genetic sexing strain (F_{60} in laboratory rearing; Lab-GSS) or males of a wild bisexual strain originating from Mauritius (F_1 in laboratory rearing; Wild-Strain). Females, who had successfully secured a mate during the 1st mating, were offered a 2nd mating opportunity 48h later by exposure to the same type of virgin males at a 1:1 sex ratio. The comparisons of numbers of matings (N) for 1st and 2nd mating were analyzed separately, with different letters indicating significant difference from each other (Tukey's test, $P < 0.05$).

In the second experiment the mating latency between Lab-GSS flies and Wild-Strain flies was also significantly different ($F_{3,572} = 27.4$, $P < 0.01$) during the first and second mating ($F_{3,248} = 32.3$, $P < 0.01$). The mating latency between Lab-GSS males and Lab-GSS females was very short and significantly different from the mating latency of all other mating combinations, which were not statistically significant among each other.

In the third experiment the mating latency in the first mating between Wild-Strain females and irradiated Lab-GSS males was similar to that of Wild-Strain females and irradiated Wild-Strain males, but in the second mating it was significantly longer with irradiated Lab-GSS males as compared to irradiated Wild- Strain males ($F_{1,20} = 4.7$, $P = 0.04$).

Discussion

Polyandry or multiple mating is common in tephritid females, particularly in species with a resource-based mating system, but also to a degree in species where the males congregate in leks and compete for females (Prokopy 1980). Refractory periods between subsequent matings of females vary from 1 day to several weeks (Tychsen & Fletcher 1971; Prokopy & Roitberg 1984; Kuba & Soemori 1988; Landolt 1994; Aluja et al. 2001; Chinajariyawong et al. 2010). Female tephritids may profit from polyandry obtaining certain indirect benefits, such as increased viability of the offspring (Arnqvist & Nilsson 2000; Opp & Prokopy 2000). However, it is not in a male's interest that the female he has mated with remates, especially in species in which the female preferentially selects the sperm of the last mating for fertilization of the eggs (last male sperm precedence). Since mating processes and mating competitiveness are fundamental components affecting the SIT, it is of paramount importance that these processes be clearly understood in order to increase the effectiveness of the SIT against the melon fly and other candidates for its use.

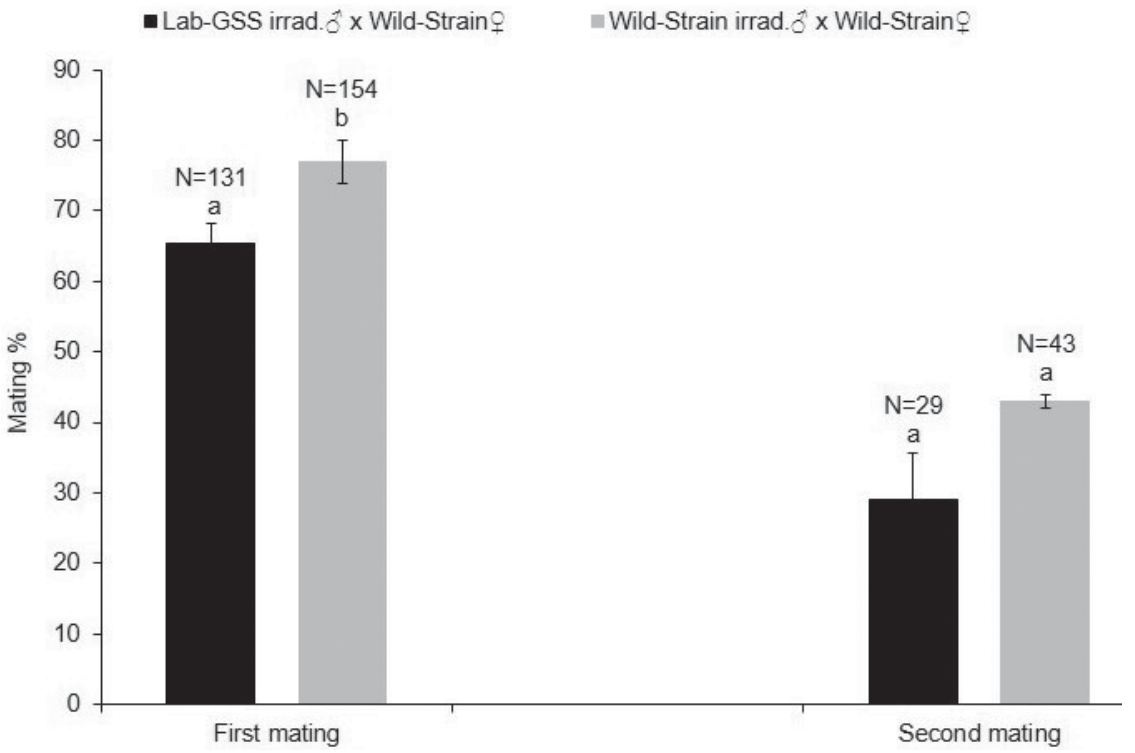

Fig 3. The percentage (mean ± S.E.) of *Bactrocera cucurbitae* females mating during a 1st and 2nd mating opportunity. The fertile wild females (Wild-Strain) were exposed in separate field cages either to virgin genetic sexing strain irradiated males (F_{67} in laboratory rearing; Lab-GSS irradiated males) or irradiated males of a bisexual strain originating from wild in Mauritius (F_4 in laboratory rearing; Wild-Strain irradiated males). Females, who had successfully secured a mate during the 1st mating, were offered a 2nd mating opportunity 48 h later by exposure to the same type of virgin males at a 1:1 sex ratio. The comparisons of number of matings (N) for 1st and 2nd mating were analyzed separately, with different letters indicating significant difference from each other (Tukey's test, $P < 0.05$).

The current studies were undertaken to understand the underlying causes of the very high remating behavior of females of a GSS of *B. cucurbitae*, in which up to 90 percent remated 48 h after the first mating under field cage experiments. This remating frequencies were significantly higher than that of females of the bisexual strain from Okinawa, Japan, whose remating frequency was tested under laboratory conditions (Kuba & Soemori 1988; Kuba & Itô 1993). If the higher remating frequency in females of the Lab-GSS is caused by the lower ability of Lab-GSS males to diminish or suppress female remating, then the incorporation of the genetic sexing strain may affect the effectiveness of SIT program.

Results of the current study confirmed that Lab-GSS females show a significantly higher remating frequency than females of either the Lab-Bisex or the Wild-Strain. However, Lab-GSS males had an equal ability to diminish or suppress remating of females of the GSS and bisexual strains. Similarly, irradiated Lab-GSS and irradiated Wild-Strain males had similar abilities to diminish or suppress the remating of females of different strains.

These results clearly demonstrated that mass-rearing had no significant effect on the mating abilities of male and female *B. cucurbitae*. Similar numbers of Lab-GSS females, Lab-Bisex females and Wild-Strain females were likely to mate with males of their strain during their first mating. However, females of the bisexual strains (Lab-Bisex and Wild- Strain) were less likely to mate with Lab-GSS males, while Lab-GSS females were mated with equal frequencies with Lab-GSS males and males of bisexual strains. Likewise irradiation had no adverse effect on the mating abilities of males; a similar percentage of females mated with irradiated males as with non-irradiated males. Moreover irradiation did not change the relative male mating abilities of the strains, i.e. the higher mating ability of Wild-Strain irradiated males than Lab-GSS irradiated males persisted..

The finding that Lab-GSS males were less accepted by females of the bisexual strains (Lab-

TABLE 1. MEAN MATING LATENCY (MINUTES ± S.E.) (TIME BETWEEN THE RELEASE OF FEMALE FLIES IN THE FIELD CAGE AND MATING INITIATION) OF SEXUALLY MATURE *BACTROCERA CUCURBITAE* FLIES OF THE GENETIC SEXING STRAIN WITH EITHER A WILD STRAIN OR A LABORATORY ADAPTED STRAIN DURING 2 SEQUENTIAL MATINGS UNDER FIELD CAGE CONDITIONS.

	Experiment 1				Experiment 2				Experiment 3	
	Mating latency in min (S.E.)				Mating latency in min (S.E.)				Mating latency in min (S.E.)	
	Lab-GSS♂ x Lab-GSS♀	Lab-Bisex♂ x Lab-GSS♀	Lab-Bisex♂ x Lab-Bisex♀	Lab-GSS♂ x Lab-Bisex♀	Lab-GSS♂ x Lab-GSS♀	Wild-Strainv x Lab-GSS♀	Wild-Strain♂ x Wild-Strain♀	Lab-GSSv x Wild-Strain♀	Lab-GSS irrad.♂ x Wild-Strain♀	Wild-Strain irrad.♂ x Wild-Strain♀
First mating	30 a (2.6)	76 b (3.7)	95 c (3.6)	68 b (4.8)	24 a (1.8)	48 b (2.6)	51 b (2.7)	46 b (3.1)	53 a (1.7)	56 a (1.6)
Second mating	46 a (4.4)	82 b (5.2)	143 c (5.9)	92 b (9.0)	38 a (2.4)	66 b (2.6)	81 b (3.7)	89 b (3.5)	87 a (2.9)	74 b (3.2)

NOTES: (1) The males and females in Experiment 1: Genetic sexing strain (F_{60} in laboratory rearing; Lab-GSS) vs. Bisexual strain originating from a laboratory adapted Mauritius strain (F_{45} in laboratory rearing; Lab-Bisex);
(2) The males and females in Experiment 2: Lab-GSS vs. Bisexual strain originating from the wild in Mauritius (F_1 in laboratory rearing; Wild-Strain); and
(3) The males and females in Experiment 3: Lab-GSS irradiated males (F_{67} and Wild-Strain irradiated males, tested in separate cages, against Wild-Strain females (F_4 in laboratory rearing) during 1st and 2nd mating.
(4) Females that mated with males of one type were exposed to virgin males of the same type 48 h after the first mating and the 1:1 sex ratio was maintained.
(5) The comparisons of treatments for 1st and 2nd mating were analyzed separately. The means followed by different letters (the comparisons of treatments among rows in each experiment) are significantly different from each other (Scheffé's test; $P < 0.05$).

GSS and Wild-Strain) is different from that of previous study (Sookar et al. 2010), in which similar mating success by GSS males and males of bisexual strains from 2 geographical regions was reported (Mauritius and Seychelles). Matsuyama & Kuba (2004) observed under laboratory conditions no mating incompatibility between an Okinawa mass-reared and a wild strain of *B. cucurbitae* from Taiwan. However differences in mating receptivity to sterile males between females from Okinawa and Ishigaki Island were observed after continuous SIT application (Hibino & Iwahashi 1991). In Hawaii, Wong et al. (1982) demonstrated that laboratory reared *Bactrocera dorsalis* (Hendel), cultured for ca. 330 generations preferred to mate with members of their own strain. Similarly, flies from a wild strain of *B. dorsalis* also preferred to mate with members of their own strain in field cage experiments. Conversely, an evaluation of the mating competitiveness of the Hawaiian *B. cucurbitae* GSS against wild males showed that the GSS males achieved, in the absence of GSS females, significantly more mating with wild females than males from the Hawaiian parent bisexual strain (McInnis et al. 2004). The discrepancies between these 2 studies may be explained by the fact that they evaluated the GSS flies against bisexual wild flies, both originating from the same region, while in this study the GSS (from Hawaii) was evaluated against bisexual strains that originated from a different region (Mauritius).

Prolonged laboratory rearing is reported to adversely affect the male's ability to diminish or suppress remating by females. *Ceratitis capitata* and *B. cucurbitae* females first mated with laboratory reared males showed a higher remating rate compared with females first mated with wild males (Kuba & Itô 1993; Hendrichs et al. 1996; McInnis et al. 2002). But in this study on *B. cucurbitae* laboratory adaptation did not have a significant effect on the frequency of female remating, because similar percentages of laboratory adapted females and wild females remated. Therefore we conclude that higher remating of the females of the *B. cucurbitae* GSS is a strain characteristic rather than an effect of laboratory rearing.

Our mating latency data showed that GSS females had the shortest mating latency compared to laboratory adapted females or wild females; and this could be an effect of laboratory rearing or a strain characteristic. Hibino & Iwahashi (1991) reported that *B. cucurbitae* wild females from Okinawa Island accepted the mass-reared males earlier than wild males, which seemed to indicate that this was due to the effects of adaptation to the artificial rearing environment. However, if the shorter mating latency was due to mass-rearing adaptation, then laboratory adapted females should have had a shorter mating latency than wild females, but this was not

the case in our experiments. Instead laboratory adapted flies displayed longer mating latencies as compared to wild flies. Furthermore, mating latency responses among Lab-GSS and Lab-Bisex flies was very variable: shortest for Lab-GSS females with Lab-GSS males, longest for Lab-Bisex females with Lab-Bisex males, and intermediate for Lab-GSS and Lab-Bisex females with either type of male. In addition a uniform response was observed for Lab-GSS vs. Wild-Strain flies: shortest for Lab-GSS females with Lab-GSS males, similar for Lab-GSS females with Wild- Strain males, and for Wild-Strain females with wild or Lab-GSS males. Thus, it seems that differences in mating latency can be attributed to the strain of origin rather than the effect of mass-rearing. The effect of irradiation was more pronounced, as it prolonged the mating latency of Lab-GSS males during the second mating with Wild- Strain females. This was also the case for irradiated Wild-Strain males, although less pronounced than in Lab-GSS males.

Earlier mating by reared males has significance for effectiveness of the SIT. For in the field the operational sex ratio at leks is biased in favor of males and the release of sterile males of the GSS in the absence of sterile females significantly increases this operational sex ratio. The earlier initiation of courtship activities by GSS males (shorter latency time) may induce receptive wild females to mate and as a consequence could decrease the probability of wild females mating with wild males. Earlier courtship of GSS males leading to earlier matings with wild females during the narrow window of courtship at dusk as shown in field cage studies in Japan (Hibino & Iwahashi 1991) would increase the frequency of sterile matings as compared to wild matings, which would make the SIT significantly more effective.

These findings have implications for the area-wide control of *B. cucurbitae* with the SIT. The high remating frequency of GSS females would be inconsequential in an operational SIT program using the GSS, because the female flies are eliminated in the production process and only the sterile males are released. The lower mating success of GSS males compared to that of wild males competing for wild females is not unlike that found with conventional bi-sexual strains mass-reared and sterilized for SIT programs. Notwithstanding the lower mating success, sterile males of the melon fly GSS were effective in suppressing the wild melon fly population in Hawaii (Mau et al. 2007). Moreover this result was achieved with a relatively low sterile to wild fly over-flooding ratio, indicating that the sterile genetic sexing strain was competitive in the field (McInnis et al. 2007). Furthermore, application of a juvenile hormone analog can accelerate the male sexual maturity in the GSS and the advantages (reduced cost of holding maturing sterile males in the release facility and males are sexually mature at the time of release) are clearly available for male only releases compared with releases of both sexes (Haq et al. 2010).

Our data reject the hypothesis that the high remating frequency of GSS females is caused by the lower ability of GSS males to suppress female remating tendency. It is therefore concluded that the high remating frequency of GSS females is a strain specific characteristic, and the GSS males' ability to diminish or suppress remating of wild females is similar to that of wild males. Our data therefore corroborate the data from the field (Mcinnis et al. 2007) and support the use of the GSS strain in SIT programs against *B. cucurbitae*.

Acknowledgments

We thank Sohel Ahmed and Vivat Wornoayporn from Insect Pest Control Laboratory for their technical assistance.

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