

Sexual Competitiveness of Vienna 4/Tol-94 ‘Genetic Sexing’ Sterile Mediterranean Fruit Fly Males in Israel

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The sterile insect technique (SIT) is used as an environment-friendly means of suppressing Mediterranean fruit fly (*Ceratitis capitata*; ‘medfly’) populations in the Arava valley of Israel. The technique depends on released sterile males effectively wresting the reproductive potential away from wild, fertile males. Studies carried out in other countries have indicated that sterile males may sometimes be of inferior sexual competitiveness in comparison with their wild counterparts and that this may inhibit SIT efficacy. In the present study, field-cage experiments were conducted to investigate the sexual competitiveness of sterile male medflies (genetic sexing strain Vienna 4/Tol-94) produced in and shipped from Guatemala, in the presence of wild males in Israel. In addition, we checked whether pre-release chilling affects their sexual success. Sterile and wild males were found to be similar in mating frequency, latency until mating, insemination probability, and duration of copulations during which no sperm were stored. There was, however, weak evidence that copulations involving sperm storage were shorter for sterile males. Chilling did not influence any element of male sexual performance. In both experiments, copulations culminating in sperm storage by females were longer than those that failed, suggesting that processes occurring early on in copulation may sometimes be the source of sexual failure. Overall, these results indicate a high standard of vigor in the sterile male medflies used in the SIT program presently followed in Israel.

KEY WORDS: *Ceratitis capitata*; medfly; field cages; mating, sterile insect technique.

INTRODUCTION

The Mediterranean fruit fly (*Ceratitis capitata*; ‘medfly’) is among the world’s most pervasive, economically damaging, and persistent pests, attacking more than 350 different types of fruit (24). For domestic markets, adequate levels of control may be attained by insecticide bait sprays, such as protein baits laced with malathion (33). However, access to valuable export markets is restricted by strict quarantine standards that may not be satisfied by traditional insecticide-based approaches. In some regions, medfly populations have been reduced to acceptable levels, or eradicated altogether, by the sterile insect technique (SIT) (12,22,34). With SIT, millions of male flies are reared in dedicated factories, sterilized as pupae by ionizing radiation (15), and then released to compete sexually with wild males (14,21,37). The ova of wild females that are fertilized by sperm of sterile males are rendered unviable, leading to suppression of pest abundance in the next generation (12,15,22). The use of ‘genetic sexing’ strains, in which females are eliminated during

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development, has greatly enhanced SIT efficiency (10,13,14). SIT is a regional approach that offers some major benefits over insecticide applications. Because the released males disperse actively, SIT reaches remote pockets of infestation, such as in residential gardens, without exposing the public to insecticides. Also, SIT may at least partially replace environmentally hazardous use of insecticides as a pest management tool. Overall, SIT poses little or no risk to non-target species in the release zone. Following preliminary trials in 1989–90 (27), a full SIT program was initiated against medflies in the Arava valley of Israel in 1997, and this program continues to the present day.

SIT relies upon released sterile males wresting the reproductive potential of wild females away from wild, fertile males and thereby disrupting reproduction of the wild population. Any reduction in mating success of released males represents a compromise of SIT success that may be compensated either by improving fly quality or by increasing release rates. Irradiation has long been known to reduce sexual competitiveness of male medflies under laboratory conditions (16). Some studies carried out in Hawaii have indicated that wild females may discriminate against sterile males (25,36,37). Experiments in Chios, Greece, gave similar results (13). Sexual ineptitude of sterile flies may result from their depauperate courtship routines (2,23,38). Concerns about the ability of mass-reared males to compete with wild males in Israel were first raised during a 1975 laboratory study (32), but there appear to have been no comparative studies involving the Israel population since that time. Sexual preferences of female medflies may vary among populations, or even within populations over time (25). Transport from rearing facilities to release zones and handling after arrival might also influence the quality of released males (5). Hence, sexual competitiveness of released sterile males must be investigated on a regional basis. That was the purpose of the present study.

Studies investigating sexual competitiveness should be carried out under conditions which, as far as possible, emulate the target insect's natural mating system (10). The medfly mating system is based on male mating aggregations known as 'leks' (19,20). During the morning, groups of up to ten males gather at certain trees and each male typically occupies the lower surface of an individual leaf as a courtship territory (18-20,30). From these sites, the males emit a pheromone from everted anal glands (1,11). Sexually receptive females are attracted by the pheromone and land on individual male's leaves, where they are courted with a series of pheromonal, acoustic, visual and vibratory signals (3,40). Field cage tests, in which leks are allowed to form on an enclosed tree, are the cheapest and simplest way to assess the mating performance of released sterile males under 'open-field-like' conditions (17). In this study, we used field cage experiments to investigate the ability of sterile males to compete with wild rivals in securing copulations and inseminating wild females in Israel. Additionally, we investigated the possibility that chilling of adult flies on the day of release reduces their sexual efficacy.

MATERIALS AND METHODS

Biological material Wild males and females were obtained from infested guava and citrus fruits collected in the vicinity of Bet Dagan, Israel. Wild flies were separated by sex within 2 days of emergence to adulthood, at which time they are still several days before sexual maturity (32,41). Sterile males (Vienna 4/Tol-94 strain) were obtained as pupae from consignments *en route* to aerial release in the Arava valley. These flies are produced at the El Pino mass-rearing facility in Guatemala, where they are irradiated (minimum absorbed

dose 150 Gy) 2 days before emergence and sent by air to Tel Aviv.

After emergence, adult flies were held (at $25 \pm 1^\circ\text{C}$, *ca* 65% r.h. and 14:10 L:D photoperiod) in 15 l plastic cages (no more than 300 flies/cage) and fed a mixture of hydrolyzed yeast and sucrose (1:2 ratio), with water provided separately. Two days before use in experiments, either the sterile or the wild flies of each replicate were marked on the dorsal thorax with non-toxic paint (4). The marking regime was randomly assigned to replicates. Marking was done with a fine brush while the flies were restrained under mesh. Flies were gently moved using an aspirator. Two different colors (red and green) were used for marking on alternate days. Flies were then sorted into groups of 35 and held in 1.5 l plastic cages with a fine mesh roof. Food and water, provided separately in cotton wool, were placed on the mesh roof, through which they were accessible *ad libitum* to the flies inside. Within 1 h before experiments, any dead or injured flies were removed and the number of healthy flies was reduced to 30 per cage. Wild males were used when 8–13 d old and sterile males were used when 5–9 d old. This difference is in accord with differences in age of sexual maturity of wild and mass-reared medflies (29,32,41).

Experimental procedures Experiments were carried out during November and December 1998. Five lemon trees (1.6 to 2 m high, 0.75–1 m diam) adjacent to the Citrus Marketing Board laboratories at Bet Dagan, Israel, were used in the field cage tests. Selected trees were separated by a distance of approximately 5 m. Each tree was enclosed with a field cage (2.9 m diam, 2 m high). Heavy black cloth was secured over the roof of each cage to filter direct sunlight.

Male flies were released into each field cage at 07:30 h, and females were released at 07:45 h. Copulating pairs were collected by gently coaxing them into 10 ml test tubes, which were then plugged with a plastic stopper to prevent escape. We recorded fly markings, as well as time and place within the field cage at which the mating pair was first detected. Copulating pairs were transferred to an adjacent room and left by a window in indirect sunlight until copulation ended naturally. We assessed copula duration and whether copulation resulted in sperm storage by females. Sperm storage was assessed by ‘squash tests’: after copula ended, the whole female reproductive system was dissected out into a drop of saline solution (0.9% NaCl) and spermathecae were squashed by pressing a coverslip onto them. Sperm were readily detected by phase contrast microscope.

Experiments were concluded at 13:00 h, at which time all unmated flies were removed from the field cages in preparation for subsequent experiments. Temperature and humidity were recorded at 10-min intervals throughout experiments, using a data logger (HOBO, Onset Computer Corp., Pocasset, MA, USA) that was placed in one of the cages. Data on average temperature and humidity recorded at 30-min intervals throughout testing are presented in Figure 1.

Mating performance tests: To assess the mating performance of sterile males competing with wild males for copulations with wild females, 30 wild males, 30 sterile males, and 30 wild females were released. Thirty-three replicates were completed over 9 days. To assess the performance of wild males in the absence of sterile males, 30 marked wild males, 30 unmarked wild males and 30 wild females were released. Seven replicates were completed over 7 days.

Effects of chilling on sterile males: To assess whether chilling (4 ha 4°C) of sterile males prior to aerial release influences their mating performance, 30 chilled sterile males (chilled from 02:30 to 06:30 h on the morning of testing), 30 unchilled sterile males and 30 wild females were released. Five replicates were completed, all on 8 December.

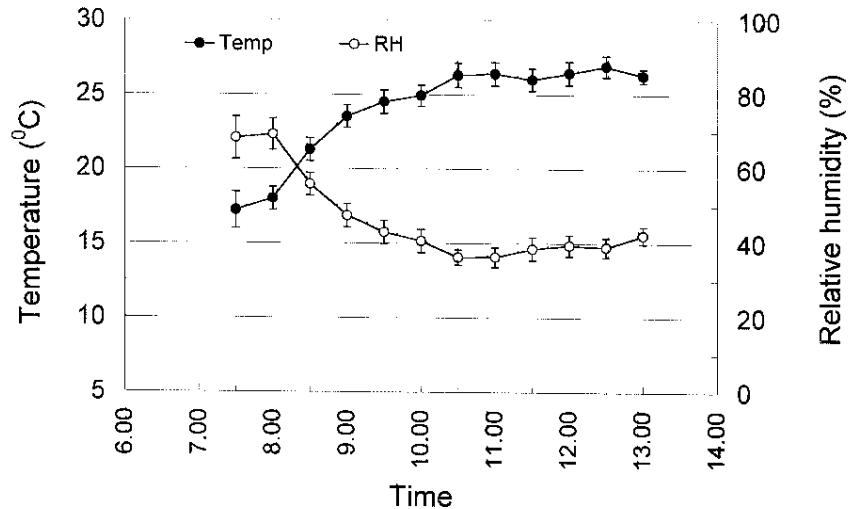


Fig. 1. Average (SE) temperature and humidity at half-hour intervals throughout the testing period.

Analyses Fly activity was measured by the Proportion of Mating (PM) index, calculated as number of mating pairs collected divided by number of females released (4). The success of sterile males at monopolizing copulations was measured by the Relative Sterility Index (RSI), calculated as matings by sterile males divided by total matings (25). Average RSI of less than 0.20 in field cage experiments with a 1:1 ratio of sterile:wild males, is considered reason for concern about the competitiveness of sterile males (17). When PM of a replicate was < 0.1 (*i.e.*, fewer than 10% of females mated), data were excluded from further analysis. Normally distributed continuous variables are presented as average \pm SE and groups are compared using Analysis of Variance (ANOVA). Frequencies were compared by Pearson's χ^2 .

RESULTS

Mating performance of sterile males Most matings of both sterile and wild males were observed on the lower surfaces of leaves, the most common site for male calling (Fig. 2). Nonetheless, there were significant differences between sterile and wild males in the frequency of copulating at the various sites assessed (5 df, $\chi^2=19.0$, $P=0.02$). Although the low frequency of copulations at sites other than leaf lower surfaces precludes more detailed statistical analysis, the most notable differences appear to be a greater tendency of wild males to copulate on the upper surfaces of leaves and a greater tendency of sterile males to copulate away from leaves altogether (Fig. 2).

The RSI averaged 0.48 ± 0.18 (three replicates were omitted due to low PM), indicating that sterile males used for SIT in Israel mate with local wild females at about the same rate as wild males under field cage conditions ($F_{1,57}=0.253$, $P=0.617$). Latency until mating did not differ between sterile and wild males (146.87 ± 6.70 and 139.39 ± 6.16 min, respectively, $F_{1,282}=0.67$, $P=0.412$). PM averaged 0.29 ± 0.18 in mating competitiveness tests and 0.26 ± 0.28 in control tests, indicating that a similar number of females accepted mates when given choices of wild only or a mixed population of wild and sterile males

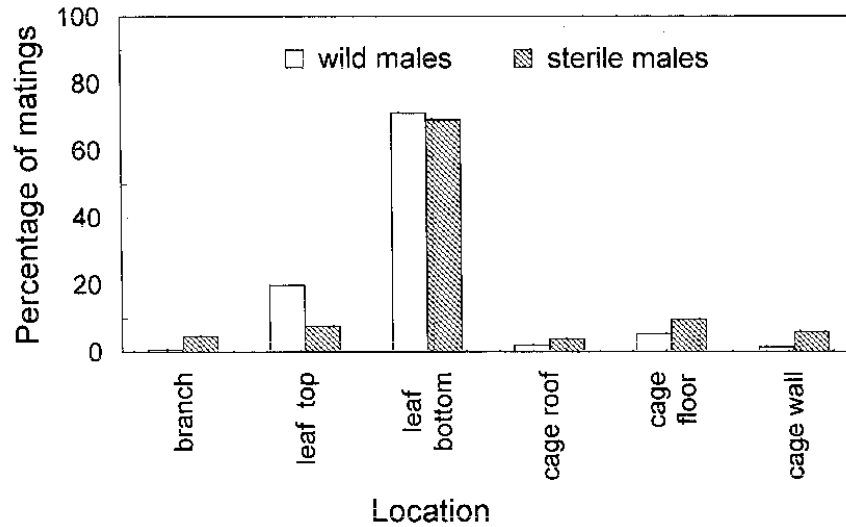


Fig. 2. Percentage of copulating pairs first observed at different sites within the field cage.

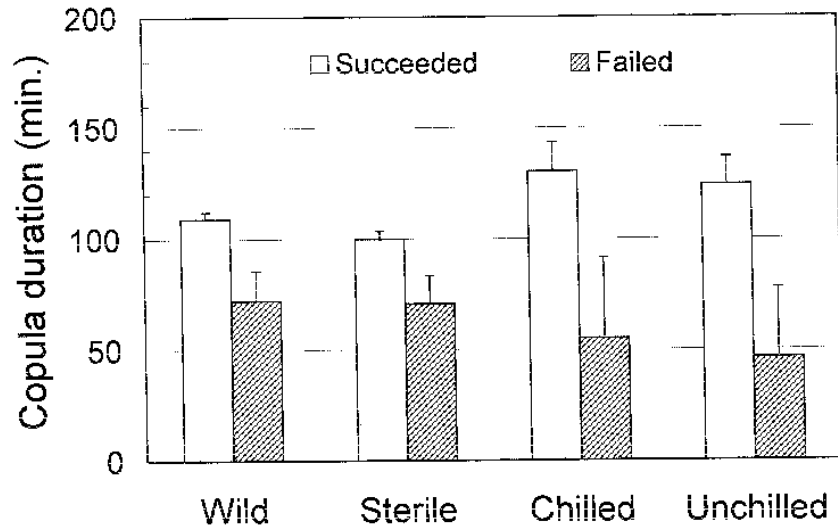


Fig. 3. Average (\pm SE) duration of copulations that did and did not culminate in sperm storage by female medflies.

($F_{1,38}=0.146$, $P=0.705$). Probability of sperm storage by mated females was similar for wild and sterile males (0.89 and 0.85, respectively, 1 df, $\chi^2=0.85$, $P=0.356$).

There was weak evidence that copulations culminating in sperm storage were shorter for sterile males than wild males (Fig. 3; $F_{1,234}=3.26$, $P=0.072$, Adjusted power=0.32), but this was not the case for copulations that did not involve sperm storage (Fig. 3; $F_{1,33} < 0.00$, $P=0.946$). Copulations involving sperm storage tended to be longer than

those that failed for both wild males (Fig. 3; $F_{1,147}=13.56$, $P<0.001$) and sterile males (Fig. 3; $F_{1,120}=6.61$, $P=0.011$).

Chilling tests Chilling of sterile males (4 h at 4°C) on the morning of testing, simulating pre-release conditions of the SIT aerial releases in Israel, did not have any detectable effect on (i) the ability of these males to secure copulations (PM=0.22±0.05, RSI as chilled/total matings=0.45±0.21, $F_{1,19}=0.628$, $P=0.451$); (ii) their latency until mating (chilled 163.33±21.77 min, non-chilled 142.67±19.87 min; $F_{1,31}=0.49$, $P=0.489$); (iii) probability of sperm storage (chilled 0.80, non-chilled 0.72; $\chi^2=0.27$, $P=0.604$); (iv) duration of their copulations involving sperm storage (Fig. 3; $F_{1,23}=0.11$, $P=0.748$); or (v) duration of their copulations that failed to involve sperm storage (Fig. 3; $F_{1,5}=0.03$, $P=0.863$). Copulations involving sperm storage tended to be longer than those that failed for both chilled males (Fig. 3; $F_{1,13}=5.66$, $P=0.033$) and non-chilled males (Fig. 3; $F_{1,15}=7.39$, $P=0.016$).

DISCUSSION

Results indicate clearly that the sterile males used for SIT management of medflies in the Arava valley are competitive with wild males for copulations with local wild females. The RSI of 0.48 attained in this study far exceeds the minimum accepted threshold for concern: 0.20 (17). High competitive standards of sterile medflies in field-cage tests have been reported also in Hawaii (42), Italy (31) and Guatemala (28). However, a similar study carried out in Argentina obtained somewhat lower RSI values of 0.26 and 0.33 for two different sterile strains tested (4).

Securing copulation is just one of many steps toward wresting reproduction from wild, fertile males and suppression of pest abundance. Male medflies that succeed in copulating may later fail to have any sperm stored by mates and therefore fail to secure fertilizations (39). Failure rates for both wild and sterile males in this study were similar both to each other and to rates for irradiated and non-irradiated mass-reared males reported in another study (35). Statistical analysis of that study's data indicates no effect of irradiation on sperm storage probability of males from a laboratory strain (fertile 85/114, sterile 102/134, $\chi^2=0.08$, $P=0.777$). However, their results do indicate that among copulations involving sperm storage, females stored fewer sperm when mating with sterile males. If number of sperm stored is associated with subsequent female non-receptivity (see ref. 26) or other reproductive benefits for males, then ultimate success of sterile males may be overestimated by our qualitative measures of mating and sperm storage frequency.

The results of this study were similar to those of other studies (35,39), in that copulations during which females did not store sperm tended to be shorter than those during which some sperm was stored. It seems that males sometimes fail to complete the complex intromission process (see refs. 8,9), perhaps being rejected in the course of cryptic female choice (6,7). There was some weak evidence that copulations involving sperm storage were shorter for sterile males. Without a detailed functional understanding of processes taking place during copulation, it is difficult to interpret such differences.

It is important to note that we tested the flies in a way that minimized exposure to harmful environmental elements up to the time of testing. There are additional factors which may play a critical role as determinants of sexual success in nature that are overlooked in field cage experiments. In particular, the ability of sterile males to survive in the hot and dry desert conditions of the release zone may be a factor to consider. Although

it is reassuring to know that those sterile males that do join leks may indeed secure a reasonable proportion of copulations and inseminations, future studies should consider just how many released males actually survive to enter the mating arena. Recent research has highlighted the fact that inability to join leks may be a crucial barrier to reproductive success for male medflies (18). Chilling of sterile males prior to aerial release does not appear to have any adverse impact on their sexual efficacy.

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