

Article

New indices for measuring some quality control parameters of the Mediterranean fruit fly, *Ceratitidis capitata* (Wied.)

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Abstract

Even though the existence of interspecific competition and competitive displacement between the Mediterranean fruit fly *Ceratitidis capitata* (Wied.) and peach fruit fly *Bactrocera zonata* (Saunders) in the last two decades in Egypt, Mediterranean fruit fly still occurs and threatens many kinds of fruits and vegetables in Egypt. The objective of this study was to estimate the sexual compatibility, mating performance and relative sterility between laboratory and wild flies of the Mediterranean fruit fly, *C. capitata* by new indices (relative mating index, RMI; relative isolation index, RII; isolation index, ISI; male relative performance index, MRPI; female relative performance index, FRPI and relative sterility index, RSI). The results revealed that different doses of gamma radiation 10, 30, 50, 70, and 90 Gy had no effect on the various parameters of mating compatibility, performance and competitiveness of lab strain males of medflies when mated with wild males. Moreover, no significant assortative or disassortative mating was observed. Therefore, we suggest that the lab strain males of medfly are compatible of mating with the wild males, at least under the laboratory conditions employed here.

Keywords sexual compatibility; mating performance; relative sterility; *Ceratitidis capitata*.

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1 Introduction

The Mediterranean fruit fly or medfly, *Ceratitidis capitata* (Wiedemann) a colorful insect of the dipteran family Tephritidae (Trypetidae), is considered one of the most destructive and important pests of edible fruits worldwide (Weems, 1981; Liquido et al., 1991, 1998; Copeland et al., 2002). It was described by Wiedmann in 1824 from the type specimen collected a board a ship in the Indian Ocean (1817).

In 1998, peach fruit fly or PFF, *Bactrocera. zonata* (Saunders) was recorded for the first time in Egypt (Agamy and Sbahia, near Alexandria). Today, it is well established in most Egyptian provinces and it causes a severe damage to a wide range of fruits (El-Minshawy et al., 1999). *C. capitata* and *B. zonata* causing a

considerable damage in many fruit species (Ghanium, 2009). The existence of medfly and PFF in the same hosts caused interspecific competition and competitive displacement between them. Most studies of competition are descriptive: an increase in the population density of one species co-occurs with a decrease in another (Connell, 1983; Schoener, 1983, 1985). Therefore, attention of both researchers and farmers tend to control the peach fruit fly only, although sticky traps and infested fruits recorded numbers of medfly in different regions of Egypt.

Over the last decades, aerial malathion-based bait-spray application has been the most common and effective control tool against exotic fruit flies (USDA, 1993). The spray consists of the organophosphorous insecticide malathion mixed with a protein hydrolysate acting as an attractant and feeding stimulant. Malathion is highly toxic to terrestrial and aquatic non target invertebrates. In fact, a malathion bait spray may disrupt a substantial portion of natural biological control agents (USDA, 2001). In Egypt, medfly and PFF populations are typically managed by trapping, partial or complete spraying, bait spraying/collection and deep burial of fallen fruits. These methods reduce but not sufficiently eliminate its populations.

The Sterile Insect technique (SIT) is a promising, environmentally friendly, methodology for control or eradication of insect pests. The effectiveness of (SIT) depends on the quality and the ability of sterile laboratory males to search for females, mating compatibility and effective competitiveness with wild males (Orozco et al., 2007).

In the present study, sexual compatibility, mating performance and relative sterility between sterile laboratory males and wild flies of the Mediterranean fruit fly, *C. capitata* was measured by new indices.

2 Materials and Methods

2.1 Wild strain

Wild flies were collected from infested mandarin fruits in El-Sharkia Governorate. Infested fruits were collected from the host plant and the ground under the trees then distributed in plastic trays (40 cm diameter) furnished with fine sand to let the larvae mature. The full grown larvae raised out "popping" to pupate in the fine sand. The pupae were daily collected and pupae in the same age were kept in separate Petri-dishes (10 cm diameter). Then pupae were kept in adult cages till eclosion. The eclosed peach fruit flies were removed from cages by aspiration.

2.2 Laboratory strain

The old strain of Medfly was maintained under laboratory conditions $25 \pm 2^{\circ}\text{C}$, 65 – 75% RH and a photoperiod of 12/12h (L/D) in the Plant Protection Department, Faculty of Agriculture, Suez Canal University. The adults were maintained in a cage (75 x 28 x 28 cm) coated with muslin fabric and placed over a plastic dish (40 cm in a diameter) filled with tap water (1 liter). Enclosed adults were provided with a diet consisted of 1 part of protein hydrolysate and 3 parts of sugar by weight. A water soaked cotton clump in a small cup served as a water source. Larvae were reared in plastic trays (15 × 5 × 3 cm) half-filled with an artificial diet. The larval diet consisted of wheat bran (1000 g), brewer's yeast (250 g), sugar (250 g), sodium benzoate (10 g), hydrochloric acid (10 ml) and water (2000 ml) (Mahmoud et al., 2013).

2.3 Sexual compatibility

Samples of pupae from lab strain were irradiated 2 days before adult emergence with doses of 10, 30, 50, 70 and 90 Gy using a ^{60}Co source (Gammacell irradiator, model 4000A) located at the Atomic Energy Authority, Gamma Irradiation Unit, Naser City, Cairo, Egypt. The gamma irradiation was carried out at a dose rate of 66 Gy/min. Gamma cell calibrated by using alanine reference dosimeter to verify the dose delivered to the pupae. 5 males, 5 females of the tested wild flies and 5 sterile males, 5 sterile females of lab strain were caged in small cage measuring 20 × 20 × 20 cm (each group in a separate cage). In order to identify the sterile males

and sterile females, few amount of fluorescent dye was applied onto the surface of the pupae, which is then transferred to the teneral adult upon emergence. Throughout the observation period the numbers of matings were recorded as wild male and female (WW), sterile male and female (SS), wild male and sterile female (WS), and sterile male and wild female (SW). The tests were conducted with 5 replicates for each dose of irradiation. Each cage contained sugar and protein hydrolysate at the ratio of 3:1 by weight and source of water.

2.4 Sexual competitiveness

To measure the relative sterility index (sexual competitiveness) between the previously prepared sterile lab males at 10, 30, 50, 70 and 90 Gy and wild males toward wild female: five competitiveness cages ($25 \times 25 \times 30$ cm) were prepared into each cage 15 sterile lab males: 5 wild males: 5 wild females were put. Each cage contained the previous adult diet. The number of matings was recorded as sterile lab male and wild female (SW) and wild male and wild female (WW).

2.5 Statistical analysis

To estimate sexual compatibility, the index of sexual isolation (ISI), male and female relative performance indices MRPI and FRPI, and the relative sterility index (RSI) (Calkins and Parker 2005) were calculated. We used the 0.25 value as variance limit for equal mating propensity in ISI, MRPI, and FRPI, and for equal competitiveness in the RSI. Data obtained in all presented experiments were subjected to an analysis of variance (ANOVA) with the honestly significant difference value calculated as Tukey's statistic at $\alpha = 0.05$ (SAS Institute, 2002).

3 Results

The results obtained from the mating compatibility test are shown in Table 1. The relative mating index (RMI) indicates the overall percentage of the couples that mated. If the competitiveness of the males were equal then 0.25 of the mating pairs would be from each of the treatment groups. All the RMI values were larger than 0.25, indicating good mating performance (FAO/IAEA/USDA, 2003). In this experiment RMI was 0.82 at 50 Gy, 0.80 at 10 and 70 Gy, 0.78 at 90 Gy and 0.68 at 30 Gy. The relative isolation index (RII) gives an indication of mating compatibility between wild and sterile laboratory strain. A value of 1 indicates random mating, with larger values indicating assortative mating. The value of RII can be interpreted as the number of sterile males that have to be employed to be equivalent to one wild male. Values normally vary between 1.5 and 5, and values consistently above 3 are a cause for concern. Results in Table 1 show values of RII range from 1.68 to 3.65. This values indicate that satisfactory levels and there was no significant difference among populations in the experiment ($F = 1.6212$; $P = 0.2079$). The index of sexual isolation (ISI) is a measure of mating compatibility between populations. The index considers the number of couples obtained for each possible mating combination, with values range from -1 (complete negative assortative mating, that is, all mating are with members of the opposite population) through 0 (random mating) to +1 (complete positive assortative mating, that is, total mating isolation of the two populations) (Fig. 1). The ISI values (Fig. 2) show good levels of compatibility between the sterile flies and the different wild populations, and there was no significant difference among populations ($F = 0.6036$; $P = 0.6645$). The relative sterility index (RSI) indicates the sexual competitiveness between two strains. Values range between 0 and +1. Zero means that wild females mate only with wild males; a value of +0.5 indicate that wild females mate indiscriminately with wild or sterile males; a value of +1 indicate that wild females mate only with sterile males. The RSI in most cases reflected the preference of wild females for wild males over sterile males. Data shows no significant difference between lab and wild flies ($F = 0.2494$; $P = 0.9066$). The male relative performance index (MRPI) is a measure of the propensity of sterile males to mate with wild females, with values ranking from -1 to +1. A value of -1 indicates that all matings were carried out by wild males, while a value of +1 indicates that all matings were

carried out by sterile males. Zero indicates that males from both populations participated equally in matings. Fig. 3 shows that the sterile males participated equally at obtaining mates with wild males and there was no differences between the populations ($F = 1.0005$; $P = 0.4304$). The female relative performance index (FRPI) is a measure of the propensity of sterile females to mate with wild males, with values ranking from -1 to +1. A value of -1 indicates that all matings were carried out by wild females, while a value of +1 indicates that all matings were carried out by sterile females. Zero indicates that females from both populations participated equally in mating. Fig. 4 shows that the sterile females were equal at obtaining mates with wild females and there was no overall differences between the populations ($F = 1.1416$; $P = 0.3656$). Data in Fig. 3 and Fig. 4 suggests that the sterile males and sterile females were participated equally in matings when competing against wild flies of medfly populations

Table 1 Relative mating index (RMI), relative isolation index (RII), isolation index (ISI), relative sterile index (RSI), male relative performance index (MRPI) and female relative performance index (FRPI) obtained from irradiated lab strain mated with wild flies.

Radiation dose(Gy)	RMI	RII	ISI	RSI	MRPI	FRPI
10	0.80	3.65 ± 2.147 a	0.281 ± 0.189 a	0.383 ± 0.370 a	-0.124 ± 0.208 a	-0.024 ± 0.143 a
30	0.68	1.68 ± 0.535 a	0.174 ± 0.089 a	0.499 ± 0.227 a	-0.231 ± 0.245 a	-0.028 ± 0.100 a
50	0.82	2.28 ± 1.156 a	0.168 ± 0.123 a	0.449 ± 0.182 a	-0.084 ± 0.268 a	-0.044 ± 0.060 a
70	0.80	2.30 ± 1.254 a	0.223 ± 0.080 a	0.316 ± 0.123 a	-0.162 ± 0.221 a	-0.192 ± 0.050 a
90	0.78	2.06 ± 0.855 a	0.187 ± 0.157 a	0.488 ± 0.197 a	0.040 ± 0.178 a	-0.053 ± 0.266 a
<i>F</i>		1.6212	0.6036	0.2494	1.0005	1.1416
<i>P</i>		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

RMI (Relative mating index) = No. of pairs collected/No. of females released.

RII (Relative isolation index) = (SS×WW) / (SW×WS).

ISI (Isolation index) = (SS+WW)-(SW+WS) / (SS+WW+SW+WS).

RSI (Relative sterile index) = (SW) / (SW+WW).

MRPI (Male relative performance index) = (SS+SW)-(WS+WW) / (SS+WW+SW+WS).

FRPI (Female relative performance index) = (SS+WS)-(SW+WW) / (SS+SW+WS+WW).

Data (± SD) followed by the same letter do not differ significantly according to Tukey’s HSD test.

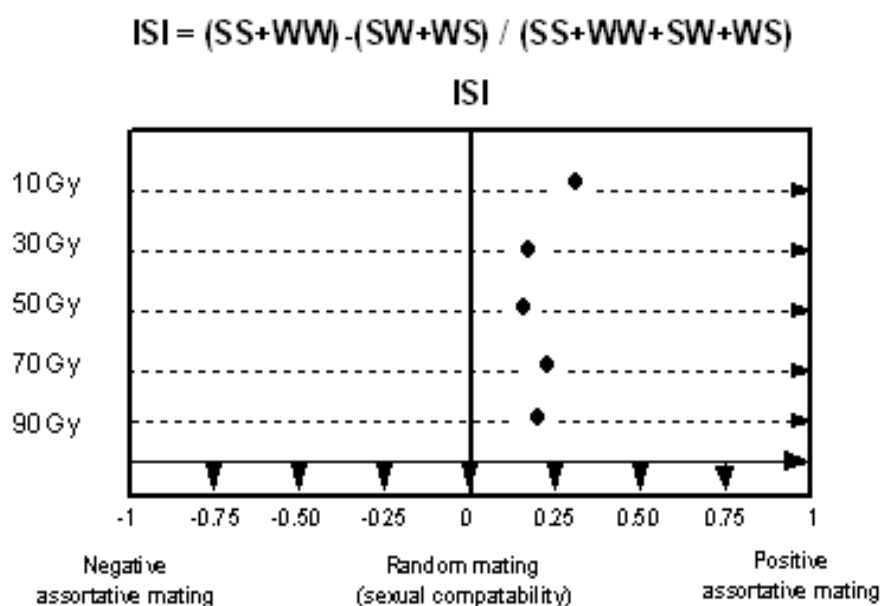


Fig. 1 Index of sexual isolation comparing the compatibility of the sterile flies with the wild flies.

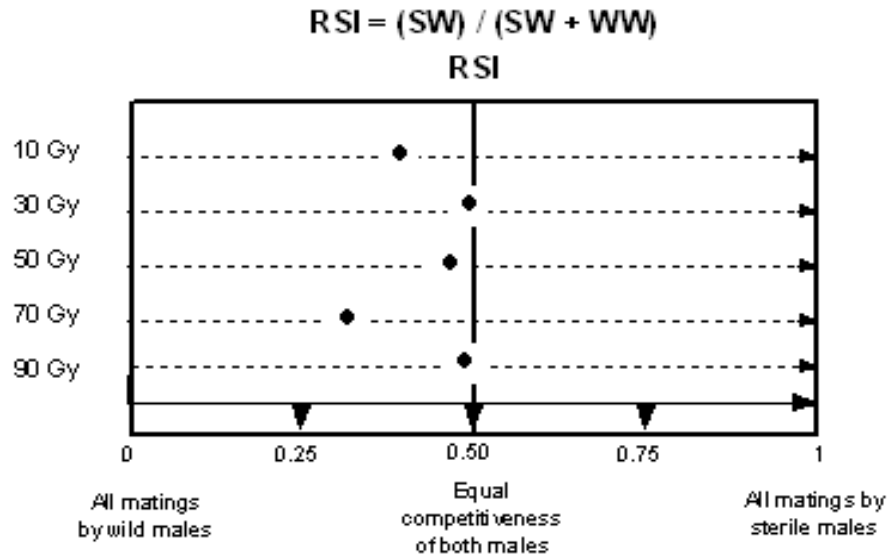


Fig. 2 Male mating competitiveness between sterile flies and wild flies.

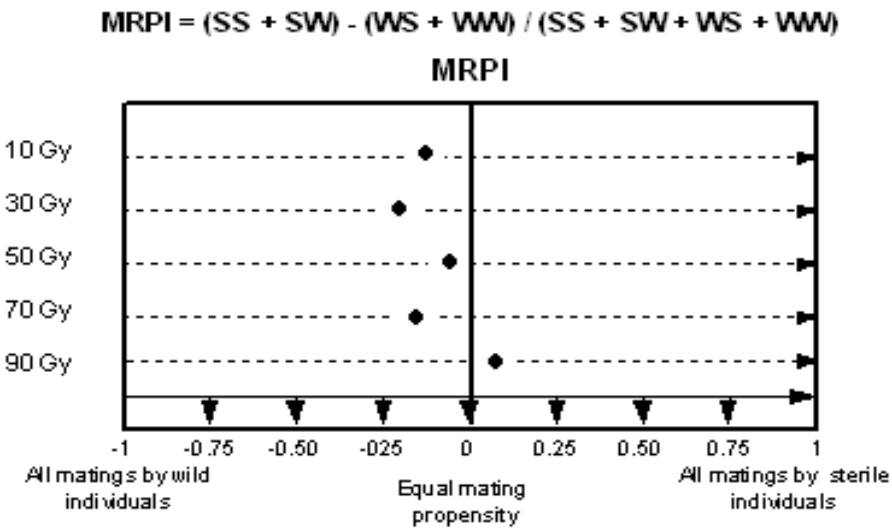


Fig. 3 Male relative performance index between wild flies with the sterile flies.

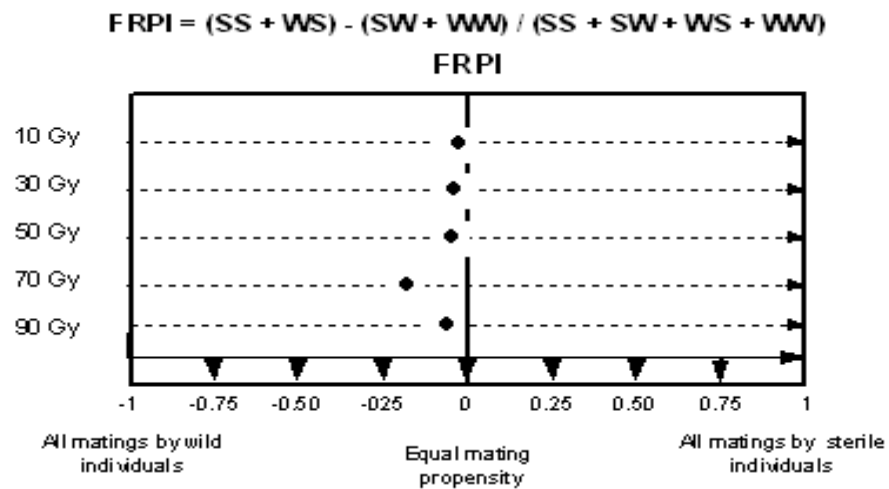


Fig. 4 Female relative performance index between wild flies with the sterile flies.

4 Discussion

The sterile insect technique (SIT) is a specific control method that may be applied in the area-wide integrated pest management of insect pests of medical, veterinary, and agricultural importance. Contrary to chemical and biological products, sterile insects are non-invasive agents rather than intrusive toxic, pathogenic or otherwise destructive entities. Therefore, the SIT alone poses a priori an exceptionally low risk to the environment (Hendrichs, 2001). SIT is widely used to suppress infestations of the Mediterranean fruit fly (medfly), *C. capitata* (Wied.) (Hendrichs et al., 2002). The success of an SIT program depends largely on the ability of sterile lab males to obtain matings with wild males.

Mating compatibility, mating performance and relative sterility are important quality control parameters that affect the performance of released sterile insects. The present study showed the indices of mating compatibility and mating competitiveness between wild and sterile population of medfly at different doses of gamma radiation from 10 to 90 Gy. Wild flies may expose constantly to the natural environmental conditions, while laboratory flies may expose to fairly stable environmental conditions. This difference causes some changes in the behavior of laboratory flies. Attempts to obtain 100% sterility in males usually reduce quality, and it will often be better to reduce the dose so as to obtain a better induction of sterility in the field females by giving a more competitive male (Toledo et al., 2004; Dyck et al., 2005).

Laboratory tests of mating compatibility and competitiveness may not reliably indicate the situation in the field (Katsoyannos et al., 1999). A much better measure can be obtained by using a field cage. Standardized procedures for field-cage operation have been defined (Cayol et al., 2002; FAO/IAEA/USDA 2003). A field cage consists of a mesh cage, 2 m high and 3 m wide, erected over a host plant or other suitable vegetation. But, it is usually not possible to detect mating pairs directly in the field. Therefore, in this present study, all experiments of compatibility and competitiveness carried out in lab-cage similar to the field cage but smaller in size (20 cm high and 20 cm wide) under laboratory conditions.

Data of mating compatibility and competitiveness indices (mating index, RMI; relative isolation index, RII; isolation index, ISI; male relative performance index, MRPI; female relative performance index, FRPI and relative sterility index, RSI) demonstrated good sexual compatibility between wild flies and mass reared lab flies. Results of RMI revealed satisfactory mating propensity between wild and sterile lab flies, because all values were larger than 0.25 of observed matings. The RII showed good indication of mating compatibility between wild and sterile lab flies. Values at all doses of gamma radiation range from 1.68 to 3.65 and indicate assortative mating. The ISI showed good mating compatibility between wild and sterile lab populations at all doses of gamma radiation (10, 30, 50, 70 and 90 Gy). Values of MRPI revealed that the sterile lab males which produced from pupae irradiated with different gamma doses (10, 30, 50 and 70 Gy) are almost equal mating propensity with wild males. However, sterile lab males produced from pupae irradiated with 90 Gy were as effective in copulating with wild females as the wild males. Values of FRPI showed that the sterile lab females are almost equal mating propensity with wild males and the wild females.

Data in the present study indicate that doses of gamma radiation had no effect on the various parameters of mating compatibility, performance and competitiveness of lab males of medflies when mated with wild males. These results can be interpreted in two ways. First, irradiation generally may have no effect on the mating competitiveness of male medflies. Alternately, irradiation may negatively affect mating ability, but its impact may vary with the general 'vigor' of the flies irradiated, being greater for individuals of low quality.

The satisfactory levels of compatibility and the good competitiveness of irradiated lab males and wild males observed in this study may encourage the application of the SIT to control *C. capitata* populations.

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