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Sterility and Mating Competitiveness of Medfly, *Ceratitis capitata* (Wiedemann)

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(Received 5 October 2000)

Abstract

The basic methodology in the determination of sterilising dosage in male medflies and mating competitiveness with the normal males was carried out in the laboratory. Application of the Sterile Insect Technique (S.I.T) by three irradiation dosages on Seib-6096 pupae results in sterility when dosage increased. A lower mating competitiveness was observed with the increase in sterility value. This value was determined from the corrected egg hatch percent. The resulting data showed that irradiation dosage of γ 10.0 Krad gave a good advantage to suppress the population in the next generation. The method suggested a good application in the control and eradication of fruit flies.

Keywords : Sterile Insect Technique, biological control, eradicate, insect pests, Medflies, sterility, mating competitiveness, next generation, insect population, irradiation, pupae, adult, sex ratio, emergence, egg hatch, pesticides.

1. Introduction

The use of pesticides to counter rapid growth of insect pests on a wide scale causes havoc to beneficial forms of life including domestic animals and mankind. One of the biological method employed in the control or eradication of a specific insect population is the autocidal control method known as Sterile Insect Technique (S.I.T). The technique is used for self destruction through the transfer or damaged reproductive material by taking advantage of the mating behaviour. The S.I.T involves the release of mass-reared sterile males into the environment occupied by the pest population. These treated insects compete with wild males resulting in sterile mating and the next generation has reduced number in population.

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The idea of using sterile insects to suppress or eradicate population of insect pests was conceived by Knippling (1955) and the validity of his theory was confirmed by the eradication of the screw worm fly, *Cochliomyia hominivorax* from the south-eastern region of the U.S.A in 1958-59 (Knippling 1960). This technique is widely used in the control of other species particularly tephritid fruit flies in the U.S.A. The melon fly, *Dacus cucurbitae* was eradicated in Rota during 1962-63 (Steiner *et al*, 1965) and the oriental fruit fly, *Dacus dorsalis* from Guam in 1963 (Steiner *et al*, 1970). Numerous other field experiments and laboratory works are carried out in many parts of the world on Medfly, *Ceratitis capitata* in Italy (Nadel and Guerrieri, 1969, Murtas *et al*, 1970), Spain (Mellado *et al*, 1974), Nicaragua (Rhode *et al*, 1971), Hawaii (Harris *et al*, 1975), Peru (Simon, 1974), Tunisia (Cheikh *et al*, 1975); on *D. cucurbitae* in Hawaii and Japan and *D. dorsalis* in Japan (Itô and Iwahashi, 1974); on the Queensland fruit fly, *D. tryoni* (Andrewartha *et al*, 1967, Monro and Osborn, 1967); and the cucumber fly, *Dacus cucumis* in Australia (Hooper, 1975 a, 1975 b, 1976); the olive fly, *D. oleae* in Greece (Tzanakakis, 1974); *Anastrepha* spp. in U.S.A (Lopez, 1970); and the cherry fly, *Rhagoletis cerasi* in Switzerland (Boller, 1974).

This paper deals with the study of sterility and mating competitiveness of the Medfly with regards to the sterilising dosage of irradiation in the control of the pests. The experiment was conducted for a period of six months at Medfly section, International Atomic Energy Agency (I.A.E.A) laboratories, Seibersdorf, Austria. Based on the resulting data the technique would be applicable in the control of the local pests and insects of medical importance.

2. Materials and Methods

The Medfly (Seib-6096) strain was used since a genetic improvement has been developed to separate the different sexes by colour of the pupae in which white pupae are designated as females and brown as males.

To produce the required amount of laboratory reared insects, maintenance of adult colony plays an important fundamental work in S.I.T. To do this two days before eclosion Medfly pupae measuring 1.5 litres in volume were spread evenly into a thin layer in an aluminium cage (200 x 100 x 20 cm). Both sides of cage are replaced by a cloth screen (terylene veil) through which females oviposited eggs. Water and adult feed (1:3 mixture of hydrolysed yeast and sugar) weighing 1.2 Kg were provided for emerging flies. The cage was placed in adult colony room in which temperature was set at $25 \pm 1^\circ \text{C}$ and relative humidity of 70%.

Three days after emergence females started to oviposit eggs through the cloth screen which were dropped into a trough of water placed under the cage. Egg collection was made for three successive days.

The collected eggs were transferred into two four-litre bottles filled with water and aeration given for 24 hours. This was done for better hatchability with a good supply of oxygen.

Before seeding larval diet was prepared. The percent weight for each ingredient used in the experiment is shown in Table 1. The diet was thoroughly mixed with hot water (80° C) after which 5 Kg weight of diet was spread evenly in each fibreglass trays (77 x 44 x 7 mm). The trays were placed in cool area to lower the temperature of the diet. When the temperature reached 25° C the diet is ready for seeding (transferring) since high temperature can kill the embryonated eggs.

Table 1. Weight of ingredients for preparation of larval diet

Ingredient	% by weight
Bran	24.0
Sugar	16.0
Yeast	8.0
Sodium benzoate	0.5
Citric acid	0.56
Hot water (80° C)	50.0

The aerated eggs measuring 3.5 ml in volume were then spread evenly into a thin layer onto the diet. This process is also known as transfer or seeding. The seeded trays were stacked in the larval rearing room in which the temperature was set at 27-28° C with relative humidity of 90%. The stack was wrapped with a plastic sheet for at least two days to keep a high humidity for the developing larvae. A larger zinc tray (120 x 80 x 15 cm) filled with bran was placed under the stack of trays. Medfly eggs begin to hatch after about 48 hours and feed on the diet.

After 7th day of transfer the larvae jumped out of the larval diet and dropped into the tray of bran for pupation. Three successive days of larval collection was done by renewal of zinc trays. The collected trays for each collection were placed separately in the pupation room where the temperature was set at 23° C with relative humidity of 70%. Pupation was completed in

two days. Using a sifting machine pupae were separated from the bran. Pupae were then spread in a ventilated tray (45 x 35 x 5 cm) made with nylon net at the bottom. Five days later they can be used for experimental purposes. Normally these pupae emerge when they are 10 days old at 23° C.

Experiment (i) Sterility Test

To determine the minimum dosage for sterilising, a sample of male flies was allowed to mate with the unirradiated females in test cages.

Selected pupae were irradiated two days before eclosion using a Cobalt 60 irradiator. Dose rates of γ 7.5, 10.0 and 12.5 Krad were tested. A sample each of 20 ml of pupae was sterilised to each dose. Samples of sterilised pupae from each treatment and a sample of unsterilised pupae were placed in separate cages (40 cm diameter x 45 cm long cylinder plastic tube), and allowed to emerge. To prevent uncontrolled mating, flies which emerged 8 hours after irradiation were used. Unirradiated (normal) pupae were also placed in a separate cage. Emerged flies were collected using an aspirator and anaesthetised with a small dose of Nitrogen gas for sexing. Sexed flies with desired ratios were combined into the test cages (17 x 11 x 11 cm) the front of cage is replaced by a cloth screen through which the females oviposited the eggs. Sterility was tested using a ratio of 1:1 (i.e. irradiated males: unirradiated females).

The test cages were held in a Quality control room with a controlled temperature of $25 \pm 1^\circ \text{C}$ and relative humidity of 70%. The flies were given water and adult food and were allowed to mate. The females oviposited the eggs through the cloth screen. The eggs were collected every second day for 10 days on moist black filter paper.

For each treatment 100 eggs were counted, placed on moist filter paper for determination of hatch. After incubation at 25°C for 5 days the number of eggs which have hatched were counted and recorded. The experiment was repeated for four times.

Calculation of sterility was determined by subtracting the egg hatch percent from 100 percent using a formula followed after Hooper (1975b).

$$St = 100 - \% \text{ EH}$$

Where "St" is the sterility value of treated males, "100" is expected egg hatch from the control and "% EH" is corrected egg hatch from specific sterility test.

Experiment (ii) Mating Competitiveness Test

To test mating competitiveness, sexed flies obtained as in experiment (i), using a ratio of 3:1:1 (i.e. irradiated male : normal male : normal female) were allowed to mate in cages. The flies were given the same treatment as in experiment (i) with 4 replicates. The competitiveness of irradiated males with those normal males for normal females were judged by the percentage of egg hatch from the tests. A formula given by Hooper (1975b) for calculating Competitiveness value ("C" value) was used.

$$C = \frac{n}{s} \times \frac{100 - Hc}{Hc - Hs}$$

Where "C" value is the competitiveness value, "n" is the number of normal males, "s" is the number of irradiated males, "100" is expected egg hatch from the control, "Hc" is corrected egg hatch from competitiveness tests, and "Hs" is the corrected egg hatch from sterility tests.

3. Results and Discussion

Results of the experiments are shown as mean values with standard errors and presented in tables and graphs.

1. The observed sterility from four replicates of pupae tested with different dosages of irradiation is shown in (Table 2 and Figure 1).

For each irradiation it was observed that an increase of gamma doses from γ 7.5 to 12.5 Krad lowered the percent egg hatch. From the view of sterility value when irradiation dose increased the sterility of male increased. Corrected average sterility showed that for γ 7.5 Krad the sterility value was $95 \pm 0.41 \%$, for γ 10.0 Krad the sterility value was $98 \pm 0.08 \%$ and for γ 12.5 Krad it was $100 \pm 0.15 \%$ respectively.

Table 2. Sterility values (and standard errors) for different replicates of 3 irradiation dosages on Seib-6096 strain.

Treatment	Rep-1	Rep-2	Rep-3	Rep-4	Average	Std.Error
Control	0 %	0 %	0 %	0 %	0 %	0.09 %
7.5 Krad	94 %	95 %	96 %	95 %	95 %	0.41 %
10.0 Krad	98 %	98 %	98 %	98 %	98 %	0.08 %
12.5 Krad	99 %	99 %	100 %	100 %	100 %	0.15 %

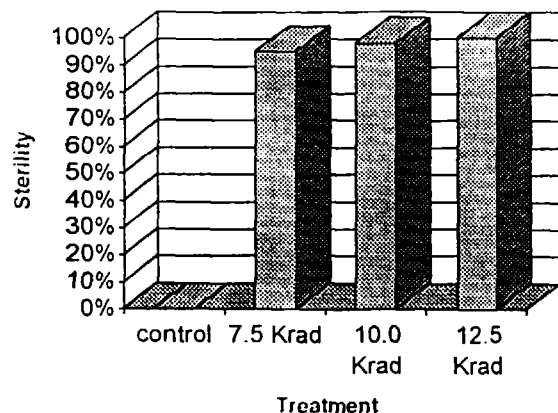


Figure 1 Comparison of male Medfly sterility

2. For the mating competitiveness test the result showed that when gamma irradiation dose increased from 7.5 to 12.5 Krad the corrected percent egg hatch increased. It showed that the mating competitiveness was lowered. The higher the dosage of sterility the lower the mating competitiveness. Results of three dosages showed that for 7.5 Krad the competitiveness value was $53 \pm 5\%$ for 10.0 Krad it was $29 \pm 1\%$ and for 12.5 Krad it was $11 \pm 2\%$ respectively (Table 3 and Figure 2). Of the three irradiation, therefore the best result could be obtained by irradiation of 10.0 Krad.

Table 3 Average mating competitiveness of Seib - 6096 for 3 irradiation dosages.

Treatment	Corrected E.H	C - value	Standard error
Control	100 %	100 %	-
7.5 Krad	67 %	53 %	5 %
10.0 Krad	78 %	29 %	1 %
12.5 Krad	90 %	11 %	2 %

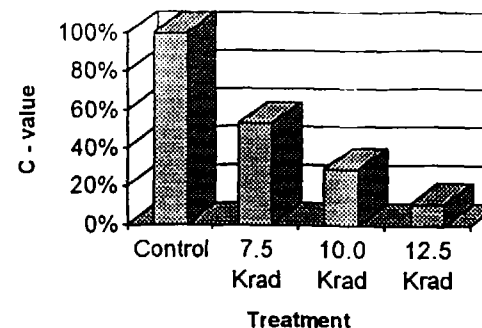


Figure 2 Comparison of mating competitiveness value

3. From the dose range it was observed that sterility of males increased from 95 to 100%, however it followed that the mating competitiveness of the males decreased with increasing dosage. Sterility and mating competitiveness leads to two opposing effects when irradiation dose was increased (Figure 3).

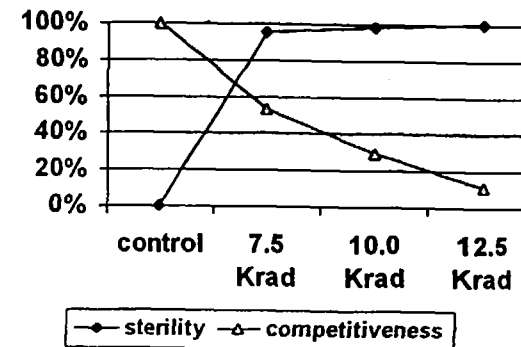


Figure 3 Comparison of sterility vs mating competitiveness

4. For the successful suppression of insect pests by S.I.T, the released insects must compete with high sterility and acceptable mating competitiveness values. A high sterility results in the suppression of population in the next generation. The higher the sterility the better the suppression of population, however the mating competitiveness is lowered resulting in the mating between normal males and females.

Of the three gamma irradiation doses in the present work 12.5 Krad showed a good sterility but lower the mating competitiveness and 7.5 Krad

has a good mating competitiveness value but the sterility is lower compared to higher doses. The present data indicated that using 10.0 Krad irradiated insects in S.I.T would give a good advantage to suppress the Medfly.

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Acknowledgement

Thanks are due to Dr. K.Fisher, Section Head, Medfly Unit, for his supervision, guidance and comments on the resulting data, and to Drs. J. Hendrich, A.S.Robinson, Head, Entomology Unit and all members of Medfly Unit International Atomic Energy Agency (I.A.E.A) laboratories, Siebersdorf, Austria, who helped in many ways during the fellowship training period.