Competitiveness of Irradiated Methyl Eugenol Fed Oriental Fruit Fly, *Bactrocera philippinensis*

by

Sotero S. Resilva and Glenda B. Obra

Philippine Nuclear Research Institute
Diliman, Quezon City
Philippines

Paper presented by the senior author in the Second Research Co-ordination Meeting of the CRP on "Quality Assurance in Mass-Reared and Released Fruit Flies for Use in SIT Programmes" held at the Mendoza Plant Protection Institute (ISCAMEN), Mendoza, Argentina, from 19 to 23 November 2001.

Year 2001

Competitiveness of Irradiated Methyl Eugenol Fed Oriental Fruit Fly, *Bactrocera philippinensis*[1]

Sotero S. Resilva and Glenda B. Obra

Philippine Nuclear Research Institute
Diliman, Quezon City
Philippines

ABSTRACT

The effectiveness of methyl eugenol feeding in the sexual competitiveness of Oriental fruit fly, *Bactrocera philippinensis* was studied. Addition of methyl eugenol concentration up to 0.5 ml per liter diet revealed no significant difference base on different quality control parameters used in the study. Results of mating tests showed high number of mated pairs were collected on flies fed with methyl eugenol both on the larvae and adult stage as compared with the untreated flies. Although no significant difference was observed between the larval and adult methyl eugenol-fed flies, the number of mated pairs slightly increased in the former than the latter in all mating tests conducted.

Introduction

Guimaras Island was recently proclaimed as the "fruit bowl" of Western Visayas. Its suitable soil and climatic conditions favor the growth and development of several fruits particularly mangoes (Manoto et al, 1997). The number of trees planted in the island was increased to one million trees in the year 2000 due to its great demand in the market. However, massive plantings and off-season induction of mangoes greatly contributed in the establishment of serious pests like fruit flies.

A pilot project to eradicate the Oriental fruit fly, *Bactrocera philippinensis* using Sterile insect technique (SIT) was conducted by the Department of Science and Technology- Philippine Nuclear Research Institute (DOST-PNRI) in collaboration with the Bureau of Plant Industry – National Mango Research and Development Center (BPI – NMRDC) in Guimaras. This is an area-wide method of insect control which involves mass-rearing, sterilization with gamma radiation and release of sterile fruit flies into the target areas to stop reproduction in the field (Obra et al, 2000). The technology was proven successfully in the eradication of melon fly in Kume Island (Iwahashi,1977) and the entire Okinawa Prefecture in Japan (Yamagishi et al, 1993).

---

[1]Paper presented by the senior author in the Second Research Co-ordination Meeting of the CRP on "Quality Assurance in Mass-Reared and released Fruit Flies for Use in SIT Programmes" held at the Mendoza Plant Protection Institute (ISCAMEN), Mendoza, Argentina, from 19 to 23 November 2001.

In other countries, application of SIT to eradicate tephritid fruit flies have not been successful. Among possible cause of failures is the reduction of sexual competitiveness of male flies by exposure to gamma rays (Holbrook and Fujimoto, 1970; Tsiropolous and Tzanakakis, 1970; Ohinata et al., 1971; Hooper, 1972). This led many researchers to develop or find solutions to regain or increase the flies' sexual competitiveness either by reducing the dose rate of gamma rays exposure or by using paraphermones or male lures.

Exposing sterile males to paraphermones before release may increase the effectiveness of SIT (Shelly and Dewire, 1994). *B. dorsalis* male oriental fruit fly fed with methyl eugenol mated more frequently and displayed high levels of wing fanning which become more attractive to females. Similar findings were observed in *B. philippinensis* males showing great advantage over wild males that were not given access to the lure (Shelly et al, 1996). Evaluation of physiological behavior of released *Ceratitis capitata* (Weidemann) on odor-baited traps had been studied, (Prokopy and Resilva, 1996) but no data relative to mating success and mating efficiency had been reported.

In this study, we investigate the effects of methyl eugenol male lure feeding in the mating behavior of male oriental fruit fly. Irradiated sexually mature males fed with methyl eugenol and males that were previously fed with the lure on the larval stage were compared in terms of mating ability with the wild population of Oriental fruit fly.

Material and Methods

A. Laboratory Flies

Oriental fruit fly obtained from the stock culture were maintained for 67 generations at the Entomology laboratory of PNRI. Two types of fruit fly methyl eugenol feeding were conducted, the larvae and adult feeding method.

1. Methyl Eugenol (ME) Larval Feeding.

Before the actual ME larval feeding was conducted, different concentrations of the lure such as 0.25, 0.5, 1.0 , and 2.0 ml per liter of diet was screened to determine its effects on the different developmental quality control parameters of OFF. Each different concentration of male lure was hand-mixed with the larval diet used in the laboratory (Rejesus et al, 1973; 1975). One hundred eggs counted in moist blotting paper squares were placed on the surface of each diet mixture scooped in plastic cups. The cups with eggs were covered with cheese cloth and placed in shelves at room temperature. After 7 days, the diet were washed and mature larvae were counted and transferred to another plastic cups containing coconut coir dust for pupation. After one week in coir dust, pupae were separated from the pupation medium and held in plastic cups until eclosion. For each diet mixture, the following data were collected in comparison with the control

lot,: 1) pupation, 2) pupal recovery 3) pupal weight and 4) eclosion. The least concentration of methyl eugenol mixed with the diet without any significant difference from the control lot based on the developmental parameters will be used as larval diet for the mating tests. Data from each study were subjected to ANOVA and the means between treatments were compared using the Duncan's Multiple Range Tests (DMRT).

2. Preparation of Methyl Eugenol Larval Fed Flies.

A concentration of 0.25 ml methyl eugenol/liter diet was not significantly different in comparison with the control lot based in all developmental quality control parameters use. This diet mixture was selected and prepared for actual feeding of larvae for the mating tests. Eggs were seeded on this diet, allowed to develop into larvae and pupated in coir dust. Two days before eclosion, the pupae were sieved, marked with rocket red fluorescent dye and irradiated using 50 Gy. Irradiated pupae were held in small cages until emergence and segregated the male and the female flies. The segregated flies were held in cages at room temperature provided with food and water prior to the mating tests.

3. Preparation of Methyl Eugenol Adult Fed Flies

In this method, newly-emerged adult flies obtained from the same batch of irradiated flies for release were sexed and placed in small cubicle cages. Segregated flies were provided with sugar and yeast hydrolysate as food. After 10 days, the flies were exposed to a cotton wick in a bottle ampoule to which methyl eugenol had been applied. The bottle ampoule were placed at the center of the cage and the flies were given access to the lure during a period of 30 minutes 2 days before mating test.

4. Preparation of Irradiated Flies (IR)

Newly-emerged male and female adults obtained from the same class of irradiated flies for shipment to Guimaras were separated and confined in small cages. The flies were provided with sugar, enzymatic yeast hydrolysate and water until mating tests. Similar holding conditions applied to ME flies were also followed in the IR flies, however, the latter was deprived from exposure to ME solution.

B. Preparation of Wild Flies (WF)

Larvae and pupal samples were collected from infested fruits obtained from Guimaras Island. These wild pupae were brought to PNRI to emerge in small cage provided with a mixture of sugar and yeast hydrolysate as food. After 20 days, the eggs were collected using a yellow perforated plastic container. A sponge wet with guava extract were enclosed within the container to stimulate the flies to lay eggs. The eggs were collected after 24 hours and the larvae were reared using papaya-brewer's yeast medium developed in Fuji. These wild flies were continuously reared for 6 – 7 generations and replaced again with a new batch of wild pupae collected in Guimaras Island. The purpose of rearing these strain is to have a continuous supply of wild adult

flies with uniform age to be used in various field cage mating tests. Since this wild strain matures late, preparation of test insects for mating experiment were done one week ahead than the laboratory flies. All the wild strain used in the entire experiment were non-irradiated and unmarked.

C. Mating Test

All mating tests was conducted in a three large 356 cm long x 185 cm wide x 182 cm high outdoor nylon screen cages located at the grounds of the Fruit fly Mass Rearing facility. Each field cage enclosed a non-fruiting guava tree whose canopy was about 1 m in diameter. Two types of fly crosses were made and set up in individual cages;

1. Simple Crosses

50 ME (A) ♂ X 50 WF♀
50 ME (L) ♂ X 50 WF ♀
50 IR ♂ X 50 WF ♀
50 WF♂ X 50 WF♀

2. Multiple Crosses

50 ME ♂ X 50 IR ♂ X 50 W F ♂ X 50 ME ♀
50 ME ♂ X 50 IR ♂ X 50 W F ♂ X 50 IR ♀
50 ME ♂ X 50 IR ♂ X 50 W F ♂ X 50 WF ♀

50 ME ♀ X 50 IR ♀ X 50 WF ♀ X 50 ME ♂
50 ME ♀ X 50 IR ♀ X 50 WF ♀ X 50 IR ♂
50 ME ♀ X 50 IR ♀ X 50 WF ♀ X 50 WF ♂

In simple crosses, pairing of male and female flies of different class were released and collected the number of mated pairs. In multiple crosses, 6 sets of different group of flies were released and observed for mating preference studies. In the latter test, mating success of larva and adult fly fed with methyl eugenol was tested in comparison with the irradiated non-methyl eugenol fed and wild flies. The number of each mated pair was recorded in one of the nine possible combinations;

ME ♂ X ME ♀
ME ♂ X IR ♀
ME ♂ X WF ♀

IR ♂ X ME ♀
IR ♂ X IR ♀
IR ♂ X WF ♀

WF ♂ X ME ♀
WF ♂ X IR ♀

WF ♂ X WF ♀

For each release event, the different fly combinations were collected in glass vials and then released into each cage between 3:00 – 4:00 pm. The flies were allowed to crawl on the tip of the vial or fly freely onto the surface of the leaves. Observations and collection of copulating pairs were done in small bottle ampoules from the onset of mating (5:30 pm) until late in the evening (9:00 pm). Individual mated pairs from each crosses were kept in a freezer and identified on the following day using a UV lamp. For each cross, we carried out a total of 3 replicates for each fly types. Individual flies were used in only one trial. All residual flies in the field cage after completion of a replicate of a treatment were removed before the next treatment commenced.

Results

1. Effects methyl eugenol larval feding on pupation, pupal recovery, pupal weight and pupal eclosion.

Pupation of Oriental fruit fly is not affected by the different concentration of methyl eugenol solution and no significant differences in the development of pupae were observed in comparison with the control (Table 1). Methyl eugenol exhibited a concentration-suppression effect on pupal recovery only at dosages of 2.0 ml per liter diet. Pupal weight and adult emergence were inhibited when the concentration of the lure was increased to 1.0 ml per liter diet. Further evaluation of the adult flies emerged in the treated diet appeared morphologically the same as the control flies. Based on this results, addition of methyl eugenol concentration up to 0.5 ml per liter diet revealed no significant difference based on different developmental quality control parameters used in the experiment.

2. Simple Crosses

Table 2 gives data on the number of mated pairs collected on simple crosses of different fruit fly groups. Adult methyl eugenol-fed flies (AME) were more sexually competitive and more attractive to female wild flies than males from any other treatments based on the high number of mean number of pairs collected (27.3 ± 4.2a). Although there were no significant differences among irradiated males fed with methyl eugenol at larval stage (LME) and irradiated non-methyl eugenol fed flies (IR), an increase in the mean number of mated pairs in the former indicates that methyl eugenol feeding affects sexual competitiveness of LME males positively. In contrast, WF males were found to be less competitive than any other treatment where mean number of pairs gathered was low (18.0 ± 6.2b).

3. Multiple Crosses

Results of multiple crosses of field cage mating experiments are presented in Tables 3a and 3b. Oriental fruit flies mate only at dusk similar to melon fly (Koyama, 1982; Kuba, et al, 1984). ME, IR and WF fly groups mated indiscriminately from 5:30

to 9:00 pm, however, peak of fly encounters and copulation attempts occurred between 6:00 to 7:00 pm similar to mating propensity tests conducted in plexiglass cages. Mated pairs collected both on larvae and adult methyl eugenol fed flies ranged from 24.3 to 41.8 %. *Copulation sites were observed on the upper and lower surface of guava leaves,* twigs and branches and on the screen of the field cages. High mean number of mated pairs and percentage matings were observed on adult males fed with methyl eugenol compared with IR and WF males (Table 3b). However, flies fed with methyl eugenol at the larval stage showed no significant difference in comparison with IR and WF male flies (Table 3a). Increase in the mean number of mated pairs gathered in methyl eugenol fed flies indicated that treated flies increases its attractiveness and competitiveness to all fruit fly females of any other groups.

Conclusion

Several researches revealed that tephritid flies for SIT programmes reduces sexual competitiveness and sex phermones to attract females when exposed to irradiation. The study reported here provides information on the effectiveness of methyl eugenol in the mating system of the Oriental fruit fly. We have shown that exposing sterile males to the lures prior to mating tests can have a significant impact on the mating ability of irradiated flies. Perhaps, certain modifications or inclusion of the lure in the larval and adult diet could be further evaluated and determine its effects on the mating behavior of irradiated laboratory flies in comparison with the wild strains.

Table 1. Pupation, pupal recovery, pupal weight and eclosion for Oriental fruit fly *Bactrocera philippinensis* larvae reared on diets with, 0, 0.25, 0.5, 1.0 and 2.0 ml methyl eugenol solution.[a]

Methyl eugenol (ml/l diet)	Pupation (%)	Pupal recovery (%)	Pupal weight (%)	Eclosion (%)
0	98.2 ± 2.1a	65.1 ± 12.6a	13.9 ± 0.6a	62.2 ± 12.7a
0.25	98.6 ± 1.8a	59.1 ± 19.5a	13.4 ± 0.6a	54.7 ± 17.4ab
0.50	98.7 ± 0.8a	58.9 ± 12.6a	13.1 ± 0.7ab	50.4 ± 13.2ab
1.0	96.9 ± 3.7a	52.3 ± 12.4a	12.2 ± 1.1b	47.4 ± 11.3a
2.0	96.3 ± 2.4a	35.9 ± 2.8b	10.1 ± 0.2c	31.5 ± 2.7c

[a]Means of 5 replicates. Means followed by the same letter are not significantly different from each other by the Duncan's Multiple Range Test.

Table 2. Mated Pairs Collected on Simple Crosses of Different Fruit Fly Groups[a]

Mating Pairs	Total No. of Pairs	Mean Number[b]	Pairs (%)
LME ♂ X WF ♀	68	22.7 ± 1.5ab	45.3
AME ♂ X WF ♀	82	27.3 ± 4.2a	54.7
IR ♂ X WF♀	65	21.7 ± 5.9ab	43.3
WF ♂ X WF ♀	54	18.0 ± 6.2b	36.0

[a]Mean of 5 replicates, LME (L) – Larvae Methyl Eugenol Fed Flies; AME – Adult Methyl Eugenol Fed Flies ; IR – Irradiated Flies; WF – Wild Flies. [b]Means followed by the same letter are not significantly different from each other by Duncan's Multiple Range Test.

Table 3.a. Data on Mating Preference between Irradiated-Methyl Eugenol fed flies At Larval Stage, Irradiated Flies and Wild Flies, of the Oriental Fruit Fly, *Bactrocera philippinenesis.*[a]

Mating Pairs	Total No. of Pairs	Mean Number[b]	Pairs (%)
ME ♂X ME ♀ IR ♀ WF ♀	247	82.3 ± 14.0a	40.0
IR ♂X ME ♀ IR ♀ WF ♀	213	71.0 ± 18.7a	34.5
WF ♂X ME ♀ IR ♀ WF ♀	157	52.3 ± 13.0a	25.4

[a]Mean of 3 replicates, ME – Larval Methyl Eugenol Fed Flies; IR – Irradiated Flies; WF – Wild Flies.
[b]Means followed by the same letter are not significantly different from each other by Duncan's Multiple Range Test.

Table 3.b. Data on Mating Preference between Irradiated-Methyl Eugenol fed flies At Adult Stage, Irradiated Flies and Wild Flies, of the Oriental Fruit Fly, *Bactrocera philippinenesis.*[a]

Mating Pairs	Total No. of Pairs	Mean Number[b]	Pairs (%)
ME ♂X ME ♀ IR ♀ WF ♀	227	75.6 ± 17.0a	41.8
IR ♂X ME ♀ IR ♀ WF ♀	184	61.3 ± 9.7ab	38.9
WF ♂X ME ♀ IR ♀ WF ♀	132	44.0 ± 18.2b	24.3

[a]Mean of 3 replicates, ME – Adult Methyl Eugenol Fed Flies; IR – Irradiated Flies; WF – Wild Flies.
[b]Means followed by the same letter are not significantly different from each other by Duncan's Multiple Range Test.

Literature Cited

Holbrook, F. R. and M. S. Fujimoto, 1970. Mating competitiveness of unirradiated and irradiated Mediterranean fruit flies. J. Econ. Ent. **63**: 1175-1176.

Hooper, G. H. S. 1972. Sterilization of Mediterranean fruit fly with gamma radiation: Effect on male competitiveness and change in fertility of females alternately mated with irradiated and unirradiated males. J. Econ. Ent. **65**: 1-6.

Iwahashi, O. 1977. Eradication of the melon fly, *Dacus cucurbitae,* from Kume Is., Okinawa, with the sterile insect release method. Prot. Ecol. 4:1-28.

Kuba, H., J. Koyama and R. J. Prokopy. 1984. Mating behavior of wild melon flies, Dacus cucurbitae Coquilett (Diptera: Tephritidae) in a field cage: Distribution and behavior of flies. Appl. Ent. Zoo. 19 (3) 367 – 373.

Manoto, E. C., S. A. Covacha, H. G. Bignayan, E.G. Gaitan, N. F. Zamora and R. P.Maranon. 1997. Integrated fruit fly management based on Sterile Insect Release Method (SIRM). Annual Report.

Obra, G. B., S. S. Resilva, R. B. Ilagan, D. M. Lazo, J. M. A. Dizon, R. E. Garcia, F. C. Isip. 2000. Integrated fruit fly management program in Guimaras Island (Phase II), Annual Report.

Ohinata, K., D. L. Chambers, M. Fujimoto, S. Kashiwai and R. Miyabara. 1971Sterilization of the Mediterranean fruit fly by irradiation: Comparative mating effectiveness of treated pupae and adults. J. Econ. Ent. **64**: 781-784.

Prokopy R. J., and S. S. Resilva. 1996. Post-alighting behavior of *Ceratitis capitata* (Diptera: Tephritidae) on odor-baited traps. Florida Entomol. 79 (3): 422-428.

Rejesus, R. S., G. B. Fernandez-Garcia and R. C. Bautista. (1973), 1975. Screening of Rice bran-yellow sweet potato combination for mass rearing the Oriental fruit fly, *Dacus dorsalis*, Hendel. Phil. Entom. 2 (5):359 – 368.

Shelly, T. E., and A. Dewire. 1994. Chemically mediated mating success in male oriental fruit flies, Bactrocera dorsalis (Diptera: Tephritidae). Ann. Entomol. Soc. America 87: 375-382.

Shelly, T. E., S. S. Resilva, M. R. Reyes and H. G. Bignayan. 1996. Methyl eugenol and mating competitiveness of irradiated male *Bactrocera philippinensis,* (Diptera: Tephritidae). Florida. Entomol. 79 (4): 481-488.

Tsiropolous, G. J. and Tzanakakis, M. E. 1970. Mating frequency and inseminating capacity of radiation-sterilized and normal flies of the olive fruit fly. Ann. Ent. Soc. Am. **63**: 1007-1010.

Yamagishi, M., H. Kakinohana, H. Kuba, T. Kohama, Y. Nakamoto, Y. Sokei and K. Kinjo. 1993. Eradication of the melon fly from Okinawa, Japan, by means of the sterile insect technique, pp. 49-60 *in*; Management of insect pests: nuclear and related molecular and genetic techniques (Proc. Int. Symp. Vienna, 1992) IAEA-SM-327/4.