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Gamma Irradiation Effect on Guava Fruit Fly, *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae)

Kessuda Puanmanee[1], Arunee Wongpiyasatid[2], Manon Sutantawong[3] and Praparat Hormchan[1]*

ABSTRACT

The effect of gamma irradiation on fruit fly pupae of *Bactrocera correcta*, from a ^{137}Cs source at various doses was investigated under laboratory conditions. The results indicated that the percentages of adult emergence, adult abnormality and male longevities were not significantly different from one another at 0, 5, 10, 15 and 30 Gy, while the sterilities of unirradiated and irradiated males were 23.85, 21.78, 59.10, 72.57 and 98.34%, respectively. Percent sterility at 5 Gy was not significantly different from the control (0 Gy). The investigation showed that mating competitiveness of the males when irradiated at 30 Gy was almost equal to that of the untreated males. The total competitiveness values of treated males were estimated to range from 1.45 to 2.09 for the three different ratios (male:normal male:normal female) of 1:1:1, 1:0:1 and 3:1:1. Observation of the melanization of the treated larvae after killing by freezing indicated that the larval color appeared to range from black to creamy white (from 0 to 30 Gy), with the degree of melanization decreasing with increasing doses. Total haemocyte counts (THCs) of the irradiated 1st instar larvae and observed in the 3rd instar larvae at 0, 5, 10, 15 and 30 Gy averaged 3,150, 2,200, 700, 550 and 350 h/mm^3, respectively. The THCs at every dose were noticed to be significantly different from that of the control.

Keywords: guava fruit fly, *Bactrocera correcta* (Bezzi), gamma radiation

INTRODUCTION

The guava fruit fly, *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae), is one of the most destructive pests in the genus *Bactrocera* (Wang, 1996) and occurs throughout most countries in Southeast Asia, including Pakistan, India, Nepal, Burma, Sri Lanka, Vietnam China and Thailand (Wang, 1996; Drew and Raghu, 2002). The fly is polyphagous with a wide host range, infesting the tropical and subtropical fruits of more than 30 plant families (Maynard *et al*., 2004). It causes great losses in fruit and vegetable production (Drew and Raghu, 2002) and is listed as a quarantine pest by most countries worldwide.

The practicability of the Sterile Insect Technique to control insect pests has been applied successfully against Tephritidae, at least on a regional scale. The irradiation process may reduce the mating performance of sterilized males (Alcagno *et al*., 2002). Boshra and Mikhaiel (2006) found that mature pupae of *Ephestia calidella*

1 Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
2 Department of Applied Radiation and Isotopes, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
3 Office of Atoms for Peace, Bangkok 10900, Thailand.
* Corresponding author, e-mail: agrprh@ku.ac.th

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(Guenée) irradiated at a dose of 1000 Gy prevented the emergence of both sexes. As reported by Ozyardimci *et al*. (2006), the rate of egg hatch in the almond moth, *E. cautella*, was 7.6% at 300 Gy, but was completely inhibited at 450 Gy. It was also observed that a low dose of 40 Gy was sufficient to suppress the egg production in females completely (Rull *et al*., 2007). The pupal age at the time of irradiation did not affect the sterility induced by gamma radiation in males (Allinghi *et al*., 2007). Nahar *et al*. (2006) also irradiated *B. cucurbitae* pupae with 30 Gy and demonstrated that the mating of unirradiated females with irradiated males did not affect egg production, but egg viability was reduced to 0.93%.

Irradiation is technically effective in the quarantine treatment for pests. In products, particularly those irradiated for quarantine purposes, it must be ascertained that any living insects will not be able to survive or proliferate in a new location. Nation *et al*. (1995a) suggested that an indicator that could be used easily for identifying irradiated insects might lie in the irradiation causing inhibition of the darkening or melanization that usually followed death or injury in a living insect. Supawan *et al*. (2005) reported the degree of melanization in non-irradiated azuki bean weevil larvae to be significantly different from the irradiated larvae.

The number of haemocytes was found to change with the level of irradiation. Tu *et al*. (2002) reported the effects of heavy-ion radiosurgery on haemocyte densities of *Bombyx mori*, with the percentage of dead haemocytes being higher for irradiated larvae than for unirradiated ones during the late 5th instar. Changes in the effect of radiation on haemocyte numbers in each instar of cotton bollworm, *Heliothis armigera*, were reported by Surisan *et al*. (2004). Irradiated at 75 and 150 Gy, the 1st, 2nd, 3rd and 4th instar larvae were found to have THCs that decreased during larval development.

The purpose of the current research was to investigate the effect of gamma radiation at various doses on the reproduction, competitiveness, larval melanization and the number of haemocytes of the guava fruit fly

MATERIALS AND METHODS

Radiation effect on mature pupae

Mature pupae (2 d before adult eclosion) at the larval stage of the guava fruit fly obtained from culture rearing on an artificial diet were held in a room with controlled temperature (25-27°C), humidity (70-80%) and a light:dark cycle of 12:12 h. They were irradiated with 0, 5, 10, 15 and 30 Gy at a dose rate of 72.38 Gy min^{-1} in a ^{137}Cs gamma irradiator (Mark I). A similar group of pupae was held as the control. Treated and untreated pupae were held under the same conditions. Data on adult fly emergence, deformation, longevity and sterility were recorded. The sterility of *B. correcta* was recorded from the following crosses of each radiation dose. Treated males (TM) were crossed with untreated females (UTF). Each treatment was replicated in triplicate, using oviposition cages with ten pairs of flies per cage per replication. The data were analyzed by analysis of variance (ANOVA) and the means compared by Duncan's new multiple range test (DNMRT), with all tests of significance at P=0.05.

Mating competitiveness

A study of the mating competitiveness of the irradiated males was undertaken. Irradiated males with 30 Gy as mature pupae, normal males and normal females were kept in oviposition cages. The ratios of irradiated male:normal male:normal female used were 0:1:1, 1:0:1, 1:1:1 and 3:1:1, respectively. Each cage was provided with an adult diet and water. The eggs were collected daily and the number of hatched eggs in each population was recorded. The competitiveness value and expected egg hatch rates were computed using Equations 1 and 2, as described by Fried (1971):

$$\% \text{ Expected egg hatch} = \frac{N(Ha) + S(Hs)}{S + N} \quad (1)$$

Where Ha = % egg hatch of normal males × normal females,

Hs = the % egg hatch of sterile males × normal females,

N = the number of normal males

S = the number of irradiated males.

$$\text{Competitiveness value (CV)} = \frac{\% \text{ Expected egg hatch}}{\% \text{ Observed egg hatch}} \quad (2)$$

Competitiveness values near 1.0 indicate full competitiveness and those higher than 0.75 indicate good competitiveness.

Assessment of melanization

After the irradiation treatment, larvae were reared under laboratory conditions and selected for evaluation of cuticle melanization. When the larvae reached the late third instar, they were placed in a freezer (-4°C) for 24 h and then removed from the freezer and placed on a sheet of white paper at room temperature for observation. Within the next hour, melanization was evaluated visually. The observations on the melanization process were made using a stereomicroscope (10x).

Total haemocyte count (THC)

The larvae of each irradiated dose were heat-fixed (60°C for 1 min) and haemolymph was bled from the abdominal segment cut with fine scissors. Haemolymph was then allowed to flow onto a clean glass slide and drawn into a Thoma white blood cell pipette, diluted to 1:100 by physiological versene (EDTA: Ethylene diamine tetra-acetic acid, 1 g:100 mL distilled water). After vigorous shaking and discarding the first three drops, the haemocyte from 1-mm squares (the four corners and the center square) were counted using haemocytometer (counting chamber). The experiment was carried out in triplicate. Data obtained for the THCs were statistically analyzed at the 0.05 level of probability and the means separated by DNMRT.

RESULTS AND DISCUSSION

Radiation effects on mature pupae

Adult emergence

Table 1 shows the numbers of adult emerging after irradiation of the mature pupae at 0, 5, 10, 15 and 30 Gy to be 97.00, 96.67, 98.33, 97.33 and 96.33%, respectively, while adult deformation levels were 0.67, 0.33, 1.00, 1.00 and 1.33%, respectively. Adult emergence and adult deformation were not significantly different from the control or from one another. Similar results for the percentages of emergence and deformed pupae were reported by Draz *et al*. (2008) who studied the effects of radiation on the peach fruit fly, *B. zonata*. With increasing gamma radiation doses, the percentages of adult emergence decreased, while those of deformed pupae increased. Resilva *et al*. (2007) demonstrated that different doses of gamma radiation (0-100 Gy) used to irradiate the pupae of *B. philippinensis* produced no significant differences in the percentages of adult emergence among those doses.

Longevity

The longevity of adult males obtained from irradiated mature pupae decreased gradually as the dose rate increased. Male longevities were 31.30, 30.05, 31.55 and 30.60 d, when mature pupae were exposed to 5, 10, 15 and 30 Gy, respectively, as compared with 37.70 d for normal males. The longevity of flies from irradiated pupae was less than those of untreated flies (the control) at every dosage (Table 1). There was no significant difference between the longevities of the control and irradiated adults. The results agreed with those of Pransopon and Sutantawong (2005), who

Table 1 Effects of gamma radiation on adult emergence, adult abnormality, longevity and male sterility of pupae of the fruit fly, *Bactrocera correcta* (Bezzi), irradiated at various doses.

Dose (Gy)	Emergence (%)	Deformation (%)	Longevity (d)	Sterility (%)
0	97.00 a	0.67 a	37.70 a	23.85 a
5	96.67 a	0.33 a	31.30 a	21.78 a
10	98.33 a	1.00 a	30.05 a	59.10 b
15	97.33 a	1.00 a	31.55 a	72.57 b
30	96.33 a	1.33 a	30.60 a	98.34 c

Means within column not followed by the same letter are significantly different at the 0.05% level, as determined by DNMRT.

reported the effects of gamma radiation at 0-80 Gy on the pupal stage of *B. correcta* and found that the percentage of survival of flies 17 d after adult eclosion did not show any significant differences.

Sterility

The effects of gamma irradiation on *B. correcta* male sterility when treated males (TM) were crossed with untreated females (UTF) resulted in sterility levels of the males irradiated at 0, 5, 10, 15 and 30 Gy of 23.85, 21.78, 59.10, 72.57 and 98.34%, respectively (Table 1). The sterility increased with increasing gamma radiation doses. The percent sterility at 5 Gy was not significantly different from the control (0 Gy), while they both differed from those of 10, 15 and 30 Gy.

The current study indicated that 30 Gy was the most suitable gamma radiation dose to obtain the highest sterility of males of *B. correcta* when applied on pupae 48 h before adult emergence. Pransopon and Sutantawong (2005) reported the dose of gamma radiation for sterilization of *B. correcta* to be 60 Gy, which gave a high percentage of sterility in males, but caused no egg laying in females. The effects of radiation on peach fruit fly, *B. zonata* at 30 Gy applied to pupae 48 h before adult emergence induced 98.60% sterility in males (Draz *et al*., 2008). Nahar *et al*. (2006) irradiated *B. cucurbitae* pupae before emergence with 30 Gy and observed that the mating of unirradiated females with irradiated males did not affect the production of eggs, but egg viability was reduced to 0.93%. Irradiation of males at a dose of 40 Gy produced 100% sterilization. However, these results highlight the need for further efforts to standardize experimental dosimetry and irradiation procedures for guava fruit fly that will provide a suitable platform for guiding future research on this serious pest.

Mating competitiveness

Male flies were irradiated with 30 Gy as mature pupae. Table 2 shows that for ratios of irradiated male:normal male:normal female of 0:1:1, 1:0:1, 1:1:1 and 3:1:1, the average egg hatches were 89.43, 0.66, 30.98 and 10.94%, respectively. The competitiveness value from the ratios 1:1:1 and 3:1:1 (irradiated male:normal male:normal female) were 1.45 and 2.09, respectively, indicating that irradiated males were fully competitive with normal males. The same observations were reported by Nahar *et al*. (2006) for *B. cucurbitae* males irradiated as mature pupae with doses of 30 Gy. The competitiveness value of different ratios (1:1:1 and 3:1:1) were 0.91 and 0.74, respectively. Katiyar and Ramirez (1969) also made similar observations and reported that gamma irradiation at 60-100 Gy applied to mature pupae had little or no effect on the mating ability of irradiated males of *Ceratitis capitata* (Wied).

The competitive mating tests gave an estimate of the mating competitiveness of the

Table 2 Mating competitiveness of irradiated males of guava fruit fly, *Bactrocera correcta* (Bezzi) with 30 Gy.

Cross ratio	Egg hatch (%)		Competitive value [2/]
TM : UTM : UTF [1/]	Observed	Expected [2/]	(CV)
0 : 1 : 1	89.43	-	-
1 : 0 : 1	0.66	-	-
1 : 1 : 1	30.98	45.04	1.45
3 : 1 : 1	10.94	22.85	2.09

[1/] TM = treated males; UTM = untreated males; UTF = untreated females.

[2/] Competitiveness value and expected egg hatch rates were computed as described by Fried (1971).

radio-sterilized males and the percentage hatching data obtained from the three ratios of treated and untreated males gave a measure of the total competitiveness of the treated males. A large number of sterilized males are usually released in order to compensate for the expected sexually reduced competitiveness caused by laboratory rearing and irradiation. The consequences of the strategies of the sterile insect release method will need much more attention and warrant thorough study.

Effect of gamma radiation on melanization process of *B. correcta* larvae

When the first instar larvae were treated with various doses (0-30 Gy), the color variation of the larvae ranged from black to creamy white. The control larvae (0 Gy) were extensively melanized and then turned black. Larvae irradiated with 5, 10 and 15 Gy showed similar melanization, but the black color developed more slowly than the control. Larvae irradiated at 30 Gy were just creamy white. Similar results were reported by Nation *et al*. (1995a), who stated that gamma radiation induced melanization and phenoloxidase changes in Caribbean fruit fly larvae, *Anastrepha suspens*a (Loew). At levels greater than or equal to 20 Gy, larvae failed to show typical melanization. Kongrat-arpon (2002) indicated that the degree of melanization in non-irradiated cigarette beetle larvae was significantly different from that in irradiated larvae, which decreased with increasing doses. The data obtained agreed also with Supawan *et al*. (2005), who reported the degree of melanization in non-irradiated azuki bean weevil larvae to be significantly different from that in irradiated larvae. The colors of the larval body appeared black, light gray and creamy white in color, when treated at 100, 300 and 500 Gy, respectively. Based on the results in the change of melanization of the guava fruit fly, *B. correcta*, it might be possible to use such information to indicate previous exposure of the insects to irradiation treatment.

Changes of total haemocyte count (THC)

The total haemocyte count (THC) from a haemocytometer grid was made on individuals of *B. correcta* where the larvae had been irradiated at the 1st and observed in the 3rd instar . For irradiation at 0, 5, 10, 15 and 30 Gy, haemocyte amounts were found to be 3,150 ± 95.74, 2,200 ± 336.65, 700 ± 191.49, 550 ± 50.00, 350 ± 50.00 h/mm^3, respectively, which resulted in increasingly higher reductions of 30, 78, 83 and 89% at 5, 10, 15 and 30 Gy, respectively (Table 3). There were no significant differences among the THCs of larvae irradiated at 10, 15 and 30 Gy with those at 5 Gy. In addition, the THCs at every dose were found to be significantly different from that of the control.

The differences in the THC between unirradiated and irradiated insects could be compared to the experimental results of Tu *et al*.

Table 3 Total haemocyte counts (THCs) and percent reduction of the 3rd unirradiated and irradiated larvae at the 1st instar stage of *Bactrocera correcta* (Bezzi) from different gamma radiation doses.

Dosage (Gy)	Mean ± S.E. of THCs (h/mm3) (% reduction)
0 (control)	3,150 ± 95.74a
5	2,200 ± 336.65b (30.00)
10	700 ± 191.49c (78.00)
15	550 ± 50.00c (83.00)
30	350 ± 50.00c (89.00)

Means within column not followed by the same letters are significantly different at the 0.05 % level, as determined by DNMRT.

(2002), involving *Bombyx mori*, which had a higher percentage of dead haemocytes for irradiated larvae than for the unirradiated ones during the late 5th instar. This also agreed with the report of Nation *et al*. (1995b), which involved gamma irradiation with 50 Gy to the 1st instar and observed in the 3rd instar larvae of the Mediterranean fruit fly, *C. capitata* and resulted in 30,000 h/mm^3 hemolymph in unirradiated larvae compared with 500 h/mm^3 hemolymph in irradiated larvae. In addition, Surisan *et al*. (2004) irradiated *Heliothis armigera* larvae at 75 and 150 Gy and found the 1st, 2nd, 3rd and 4th instar larvae had reduced THCs during larval development.

CONCLUSION

There were no significant differences in emergence, deformation and longevity of male flies irradiated as pupae with 0, 5, 10 15 and 30 Gy levels of gamma radiation. The sterility levels of irradiated males mating with untreated females were significantly different between 0 Gy and other doses, except at 5 Gy. Competitiveness values for ratios of 1:1:1 and 3:1:1 of irradiated males with 30 Gy indicated full competitiveness of the irradiated males. The melanization in irradiated larvae decreased with increasing doses and could be used for indicating irradiation treatment with levels greater than or equal to 30 Gy. The total haemocyte count decreased with increasing doses.

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