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Effect of Gamma Irradiation on Coconut Leaf Beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae)

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The effect of irradiation on the fecundity, egg hatchability, and longevity of the coconut leaf beetle (CLB) – ***Brontispa longissima*** **(Gestro) – was studied using different doses of gamma radiation ranging from 20–50 Gy for pupa and adult males, and 20–40 Gy for adult females. The fecundity and fertility of adults decreased with irradiation dose. The pupae were more sensitive to irradiation than the adults based on LD_{50} values after 14 d. Adult females were more sensitive to irradiation than males based on fecundity and egg hatchability. No eggs were hatched at 40 Gy in irradiated females (IF) mated with unirradiated males (UM) and at 50 Gy in unirradiated females (UF) mated with irradiated males (IM), albeit with a significant effect on longevity at this dose. Using 45 Gy for mating competitiveness studies at a ratio of 1:15:1 (UF: IM: UM), wild females' acceptance of IM was high based on the relative sterility index (RSI) value of 0.66.**

Keywords: egg hatch, ionizing radiation, life span, mating, SIT, sterility

INTRODUCTION

CLB, *Brontispa longissima* (Gestro), is one of the most serious insect pests of coconut in Southeast Asia. CLB is a leaf-scraping insect pest, particularly of palms, that was introduced to the Philippines most probably via the ornamental trade route. Its life span from egg to adult is close to a year and occurs in unopened leaflets in overwhelming numbers. Severely damaged leaves appear burned or scorched as a result of larval and adult feeding (Zipagan 2008). Heavy infestations by the beetle turn the leaves brown and decrease fruit production. Successive severe defoliation can lead to the death of the tree.

The Philippines has the second largest coconut population in the world, following Indonesia (Magat 2008). In 2005, CLB – which has been expanding in Southeast Asian countries – was first reported to bring damage to coconut plantations in the Philippines (Liebregts 2006, Zipagan 2006). The pest was probably introduced to the Philippines in early 2004 (Zipagan 2006) but its damages on coconut plants were only noticed in 2005. This pest seemingly continues to spread westward and, thus, countries in South Asia (Singh and Rethinam 2005) – including India, Sri Lanka, and Bangladesh – is at great risk of invasion. Since there are a large number of coconut industries in these countries, the pest incursion would be catastrophic (Nakamura *et al.* 2006). The entire life cycle of CLB takes place in the unopened spear leaf. Eggs are laid in grooves chewed into the leaflets and covered by excreta. Egg incubation is about 3–7 d and the larvae undergo six larval instars on a period of 23–54 d. The pupal period is 4–6 d. The whole cycle from egg to adult takes 31–67 days (Singh and Rethinam 2005).

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Current control methods include the use of contact and systemic insecticides. However, the problem with insecticide resistance and residues is not far from remote with chemical control.

The sterile insect technique (SIT) is an environmentally-friendly insect pest control method. SIT involves mass-rearing, sterilizing, and releasing large numbers of insects to reduce or eliminate subsequent generations of populations of the target pest (Knipling 1979). Although there are numerous studies on the sterility doses of different stages of other Coleopterans, the effect of gamma irradiation on CLB has not been documented. The use of SIT for CLB is promising since the insect can be easily reared using coconut leaves. Moreover, the beetles are mostly confined to younger leaves of the coconut and do not transfer from one plant to another quite easily. This paper reports on the effects of ionizing radiation on egg fertility, survival, mating frequency, and competitiveness of CLB. This information will be useful for the possible use of radiation-induced sterility in CLB to manage this pest.

MATERIALS AND METHODS

Source of Test Insects

Rearing of CLB collected from coconut seedling and young palms (< 10 yr) in Polangui, Albay (13°17'37.56"N, 123°29'03.8"E); Tiwi, Albay (13°27'24.95"N, 123°40'46.91"E); and from the Philippine Coconut Authority – Albay Research Center (PCA-ARC, 13°13'00.4"N, 123°34'25.28"E) was done at the PCA-ARC biocontrol laboratory using coconut leaflets cut into pieces of 5–7 cm in length. Gravid adult females were placed in a plastic box (91 cm x 61 cm x 36 cm) containing small pieces of leaves for oviposition. The adults were transferred to a new container box with fresh leaves every 2 d. Larvae that hatched from eggs were transferred into a box (11 cm x 16 cm x 6 cm) at 120 larvae per container, with small pieces of leaves that would be replaced every 3–4 d. The pupae were kept in boxes with new leaves to serve as food for emerging adults. Rearing was done at 28–30 °C.

The semi-mature larvae of CLB were shipped to the DOST-PNRI, Quezon City. The larvae were kept in plastic containers with enough coconut leaves as food. The pupae were then separated and kept in new plastic containers for adult emergence. Upon emergence, separation of sexes was done under a dissecting microscope following the diagnostic characters described by Ayri and Ramamurthy (2012) – the sixth abdominal sternite is visible in males and invisible in females. Adult males were held separately from adult females. Adults were confined in perforated plastic cups (100 mL capacity) provided with coconut leaves (3–4 cm) and were allowed to lay eggs. Eggs were collected and were placed in Petri dishes with young coconut leaves, serving as food when the eggs hatched into larvae. The test insects were held in the laboratory with a mean temperature of 26.4 ± 0.03 °C and a relative humidity of 87.5 ± 0.15%.

Irradiation of CLB

Irradiation was carried out using the Gammacell 220 irradiator with Co-60 source (MDS Nordion, formerly Atomic Energy of Canada, Limited; Ottawa, Canada). Fricke dosimeters were used to determine the actual absorbed dose.

One- to three-day-old adult males and four- to five-day-old pupae were irradiated with doses ranging from 20–50 Gy, whereas one- to three-day-old adult females were irradiated with 20–40 Gy. Untreated pupae or adults of the same age served as the control. After irradiation, both the irradiated and unirradiated adults and pupae were brought to the DOST-PNRI holding laboratory for evaluation of treatment. The pupae were allowed to develop into the adult stage. IM specimens were mated with UF, IF with UM, and UM with UF (as control).

Irradiated and unirradiated adults were initially confined in glass vials (1 male: 1 female) and were provided with coconut leaf as food. They were later transferred to plastic cups when actual mating was observed. The number of eggs laid by a mated female was counted and collected daily. The eggs were transferred to fresh coconut leaves and placed in clean Petri dishes to determine the hatch rate. Adult mortality was also recorded daily during feeding and egg collection.

Mating Tests for Competitiveness of IM CLB

Male CLBs irradiated with 45 Gy were first tested in cylindrical cages (10 cm diam. x 14 cm long) using different ratios of UM, IM, and UF varying only the number of IM. UM or IM specimens were marked interchangeably in different replications with white water-based paint using a soft brush on the distal part of the elytra to distinguish the IM from UM. Three trials were made for four different ratios of UM: IM: UF (1:1:1, 1:5:1, 1:10:1, and 1:15:1).

Using the most competitive ratio of (1:15:1), the mating frequency and duration were determined using a netted tent-like cage (2 m^2 and ca 2 m high) containing a potted coconut plant placed at the center of the cage. Adult males were released earlier than females. The adults were allowed to mate and mating activities were observed inside the cage upon release of the adult females. The durations of mating were recorded per day of observation on four occasions that were considered as trial periods.

The RSI, an overall index of the mating competitiveness of sterile males (FAO/IAEA/USDA 2003), was used. It is represented by the formula:

$$RSI = \frac{SW}{SW + WW}$$

where: SW = sterile male x wild female matings; WW = wild male x wild female matings.

The RSI was originally used to determine the male competitiveness of sterile fruit flies. The RSI indicates the proportion of unirradiated wild females mating with sterile males. Values range between 0 and +1. Zero (0) indicates that wild females mate only with wild males, a value of +0.5 indicates that wild females mate indiscriminately/ equally with wild or sterile males, and one (+1.0) indicates that wild females mate only with sterile males.

Statistical Analysis

Tests of assumptions for normality and homogeneity of variances were first performed on raw and log-transformed data. Kruskal-Wallis non-parametric test was first used to analyze non-normal data. Multivariate analysis of variance (ANOVA) was also used since this test is robust to some deviations from normality. Games-Howell post hoc tests were performed for data with unequal variances. All analyses were done using SPSS ver. 17 (IBM Corporation, Armonk, NY) and Minitab 17 (Minitab Pty Ltd, Sydney, Australia).

Separate analyses were done for IF mated with UM and IM mated with UF. Both parametric and non-parametric tests reached similar decisions on differences among treatments on the longevity of males and females. Data on irradiated pupae were subjected to the same tests as adult IMs and IFs.

ANOVA was used to test the significant differences in the irradiation tests on male *B. longissima* at 45 Gy and mating competitiveness studies using different ratios. Significant differences between treatments were determined by Tukey's honestly significant difference (HSD) for further analysis using SAS University Edition. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

In terms of mortality, pupae of CLB were more sensitive to irradiation than the adults, as shown by lower LD_{50} values obtained after 14 d for pupae (38.2Gy) compared with those of the adults (45.5Gy), respectively. Since CLB pupae were more sensitive to irradiation, mating tests for competitiveness were only performed using irradiated adults. Generally, increasing the dose showed increasing mortality (Table 1). There was no significant difference in longevity between control (untreated) and pupa treated with 20 Gy. However – in terms of the number of eggs laid per female and the number of eggs that hatched (F1) – fecundity was significantly reduced starting at 30 Gy, although hatchability was reduced at 20 Gy. In this study, sterility is expressed in terms of the inability of the eggs to hatch caused by the mating of the unirradiated females with males emerging from the irradiated pupa. A dose of 50 Gy caused complete sterility in adult females from irradiated pupa (Table 2).

The results obtained in the study are in close conformity with those from Prabhu *et al.* (2010) who demonstrated that younger stages of red palm weevil, *Rhynchophorus ferrugineus* Olivier, are more sensitive to irradiation and that the pupa is more sensitive than adults. They

Table 1. Adult mortality (%) at different doses and lethal doses (LD) of irradiated male pupa and male adult of *Brontispa longissima* mated with UF at 14 d after treatment.

Dose	Pupa		Adult	
	No. of pairs	Mortality (%)	No. of pairs	Mortality (%)
Control	27	0	27	0
20	29	0	29	3.1
30	28	21.4	28	3.3
40	25	60.0	25	11.1
50	31	80.7	31	47.5
LD_{50}	38.2 Gy (35.0–41.6)		45.5 Gy (42.7–51.2)	
Slope ± SE	9.1 ± 1.4		5.8 ± 1.4	

Table 2. Longevity, fecundity, and egg hatchability of adult CLB irradiated at pupal stage[a].

Dose (Gy)	No. of pairs	Male longevity[b]	Female longevity[b]	No. of eggs laid per female[b]	Egg hatch (%)[b]
Control	27	316.30 ± 19.43 a	290.81 ± 22.24 a	83.81 ± 7.10 a	82.96 ± 1.96 a
20	29	255.34 ± 15.85 a	325.69 ± 17.08 a	97.69 ± 7.94 a	29.83 ± 3.57 b
30	28	139.11 ± 23.07 b	281.32 ± 20.34 a	52.14 ± 9.11 b	1.80 ± 1.28 c
40	27	47.48 ± 13.75 c	325.30 ± 17.21 a	26.67 ± 8.97 bc	3.71 ± 3.72 c
50	31	13.71 ± 3.18 c	284.87 ± 18.47 a	0.03 ± 0.03 c	0 c

[a]Adult male emerging from irradiated pupa was mated with UF.
[b]In a column, means followed by a common letter are not significantly different at 5% by Tukey's post hoc test.

demonstrated that irradiation of early, mid, and late stages of pupae and of one-day-old adults at 15 Gy resulted in 98.0%, 73.3%, and 54.8% mortality in pupae, respectively, and 23.8% in adults.

Adult CLB was quite long-lived, typical of the order Coleoptera. The longevity of adult CLB is similar to mango pulp weevil, *Sternochetus frigidus* (F.) (Obra *et al.* 2013, 2017). The longevity of control samples (unirradiated CLB adults) in this study was around 9 mo, which is about four times longer than the report of Giang and Nakamura (2009). In addition, the number of eggs laid per female was also higher among the control samples. This could be probably due to the difference in the adult food used, but may also indicate that the conditions in the laboratory were suitable for the mating and survival of CLB.

Table 3 shows the longevity of adults, fecundity, and egg hatchability of IM and mated with UF CLB. Mating involving adult males irradiated at different doses did not result in acute mortality. The doses used were not high enough to cause acute mortality but enough to prevent reproduction. Eggs were laid by females throughout their life span. Fecundity is reduced with an increasing dose of radiation but only significant at 45 Gy, not 40 Gy. The number of eggs laid by UF mated with IM (UF x IM) was significantly lower compared with the unirradiated pairs (UF x UM). A significantly low number of eggs was laid by females starting at 40 Gy. At this dose, it was observed that only one larva was able to develop into a pupa but it emerged as a non-viable adult. At 45 Gy, one hatched larva died and failed to develop further. Hatching rate decreased with increased irradiation dosage. A decrease in longevity in males of CLB was also observed with an increase in the dose of irradiation. Complete sterility was achieved at 50 Gy while longevity was significantly reduced. Hence, adult males irradiated with 45 Gy were used for mating competitiveness study.

In SIT, the focus is on the sterility of irradiated males since sterile males are the ones released in the target area to mate with their female counterparts. However, in this study, adult females were also treated with different doses and mated with unirradiated males to determine their sensitivity to gamma irradiation. Results showed that, among IF, a significant effect on egg hatchability was observed starting at 20 Gy dose as compared with IM. From 32 eggs laid per female irradiated with 20 Gy dose, it was significantly reduced to less than five eggs per female starting at 30 Gy as compared to 68 eggs laid per female in the control. It was also observed that most of the eggs laid by IF were deformed. Total sterility was achieved at 40 Gy dose (Table 4). Our findings agree with previous studies on Coleoptera (Brower 1973, Brower and Tilton 1985) showing that females require lower doses for radiation-induced sterility than males except for *Anthrenus flavipes* Le Conte (Brower and Tilton 1985) and sweet potato weevil (Sharp 1995) that require higher doses for female. This is important since the separation of sexes among insects is not easy and mixing irradiated

Table 3. Longevity of adults, fecundity, and egg hatchability of CLB (IM x UF) and control (UM x UF).

Dose (Gy)	No. of pairs	Mean longevity (d ± SE)[a]		No. of eggs/ female (± SE)[a]	Egg hatch % (± SE)[a]
		Male	Female		
Control	40	272.7 ± 19.2 a	325.1 ± 16.1 a	93.6 ± 6.0 a	83.8 ± 3.3 a
20	32	185.4 ± 16.0 b	211.2 ± 19.7 ab	67.1 ± 7.0 a	43.5 ± 4.3 b
25	43	189.7 ± 14.8 b	248.5 ± 16.4 ab	69.1 ± 6.3 a	28.1 ± 3.6 c
30	30	191.2 ± 14.5 b	242.3 ± 19.3 ab	66.8 ± 7.7 ab	17.0 ± 4.8 cd
35	44	1164.4 ± 15.4 bc	264.8 ± 14.2 ab	86.7 ± 5.3 ab	5.6 ± 2.1 de
40	36	149.2 ± 15.3 bc	261.7 ± 15.2 ab	79.5 ± 6.8 ab	1.9 ± 1.7de
45	42	104.2 ± 14.1 cd	241.0 ± 19.1 ab	44.6 ± 8.2 b	0.09± 0.09e
50	40	35.3 ± 8.4 d	189.0 ± 22.1 b	9.5 ± 4.4 c	0 e

[a]In a column, means followed by a common letter are not significantly different at 5% by Tukey's post hoc test.

Table 4. Longevity of adults, fecundity, and egg hatchability of CLB (UM x IF) and control (UM x UF).

Dose (Gy)	No. of pairs	Mean longevity (d ± SE)[a]		No. of eggs/ female (± SE)[a]	Egg hatch % (± SE)[a]
		Male	Female		
Control	18	223.8 ± 32.8 a	203.2 ± 35.2 a	67.5 ± 16.1 a	60.9 ± 11.2 a
20	18	219.8 ± 35.5 a	231.8 ± 32.0 a	32.2 ± 5.5 b	0.8 ± 0.5 b
30	18	274.6 ± 27.9 a	248.3 ± 33.2 a 171.1 ± 34.7 a	3.7 ± 1.0 b	0.8 ± 0.8 b
40	18	211.2 ± 27.8 a		4.9 ± 2.1 b	0 b

[a]In a column, means followed by a common letter are not significantly different at 5% level by Games-Howell post hoc tests for data with unequal variances.

males with fertile females during the release in the field is sometimes unavoidable in the SIT program.

Mating tests for competitiveness were done using males irradiated with 45 Gy since there was a significant effect on adult longevity at the highest dose (50 Gy) used and, at 45 Gy, hatched larva failed to develop further. Mating activities were observed in the cage upon release of the adults. When male and female CLB came in contact, the male held and mounted on the female, extended its aedeagus towards the abdominal tip of the female, and mated. Sometimes, the female refused to mate and crawled away from the male. It was observed that the male only proceeds to mate with the female after touching the female with its antennae or forelegs.

Using the small cylindrical cages, the RSI values of the irradiated males ranged from 0.17–0.39. Among different ratios, RSI increased with an increase in the number of sterile males, albeit no instance of "not significant" (Figure 1). The highest RSI was obtained in a 1:15:1 ratio; however, the RSI values were still below 0.5 (0.5 indicates indiscriminate mating). The RSI values obtained from the remaining ratios suggested UF favored mating with UM and, therefore, IM was less competitive. Laboratory tests of mating compatibility and competitiveness may not reliably show the same condition in the field (Katsoyannos *et al.* 1999). Moreover, low mating competitiveness can be compensated by increasing the release ratio of the irradiated or sterile males to wild males. For example, in Guatemala, releases of 100:1 sterile: wild male Mediterranean fruit flies – *Ceratitis capitata* Wiedemann – resulted in a significant decline in fertility of a wild population (Rendon *et al.* 2004). However, increasing the ratio of sterile to wild male releases would mean an increase in the production cost in the rearing facility. Meanwhile, a release ratio between 7:1 and 10:1 of sterile to wild male tsetse fly – *Glossina palpalis gambiensis* Vanderplank – was indicated to be competitive to wild type males for mating with target females in Burkina Faso (Sow *et al.* 2012).

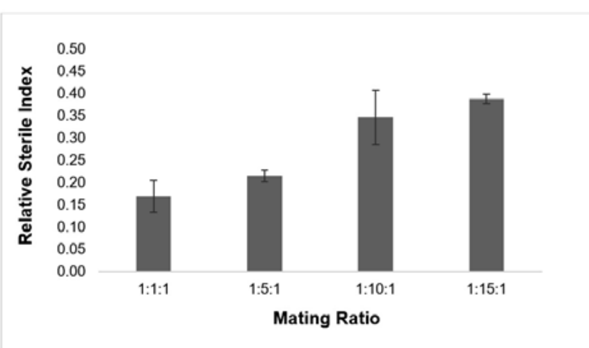

Figure 1. RSI of male CLBs irradiated with 45 Gy at different ratios of UM, IM, and UF using small cylindrical cages.

The release of UM, IM, and UF CLBs at 1:15:1 ratio in a big cage containing potted coconut plant resulted in a higher frequency of mating (Figure 2). At this ratio, mating between IM and UF was equally competitive with UM and UF. This might be due to a larger confinement area exposed to the unirradiated female and to more subtle effects of irradiation in large cages, which were not apparent in small cage experiments. Such effects may include failure to locate mates and achieve insemination under semi-field conditions (Maiga *et al.* 2014). Meanwhile, the average duration of mating of the female and male beetles was almost the same for both IM and UM, which is around 6 min (Figure 3). This may indicate that the mating ability or fitness (frequency of mating) and sperm transfer (duration of mating) of the irradiated males may not be significantly affected at 45 Gy. The RSI value computed for 1:15:1 ratio of 0.66 ± 0.11 showed a high acceptance by wild females of irradiated males, indicating the sexual competitiveness of irradiated males with wild males.

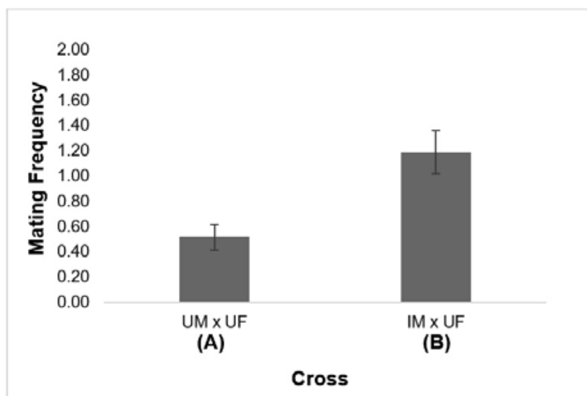

Figure 2. Mating frequency of A) UM and UF, and B) IM at 45 Gy and UF among CLBs using 1:15:1 ratio (UM: IM: UF) in cages with potted coconut plant.

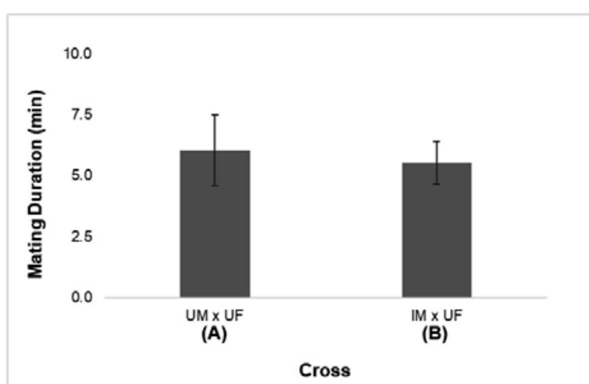

Figure 3. Duration of mating per day of UF crossed with A) UM and B) IM at 45 Gy among CLBs using 1:15:1 ratio (UM: IM: UF) in cages with potted coconut plant.

CONCLUSION

The study demonstrated that the reproductive ability of CLB was significantly decreased with increasing dose of gamma radiation at the pupal and adult stages. For SIT, it is recommended to irradiate the adult stage since they are less sensitive to irradiation than the pupal stage using 45 Gy. However, it is imperative to study how adult longevity and survival of irradiated adults could be improved, and also to demonstrate the mating competitiveness of irradiated males with wild males over wild females under semi-field conditions using also other competitiveness tests. Another good study in the future following this study as a baseline is simulation research demonstrating the potential rate of decrease of CLB populations over the next generations with a release (or scheduled batches of release) of irradiated males in a target area.

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