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Effect of Gamma Irradiation on Egg Hatchability, Adult Survival and Longevity of the Mango Pulp Weevil, *Sternochetus frigidus* (Fabr.)

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The effect of irradiation on egg hatchability, adult survival and longevity of *Sternochetus frigidus* (Fabr.) was studied using different doses of gamma radiation. Irradiation lowered egg hatchability, longevity and survival rates of *S. frigidus*. Response of males and females in terms of longevity and survival rates differed at sub-efficacious and efficacious doses. The study shows that the use of phytosanitary irradiation against *S. frigidus* results in a lowered risk that adults would be detected by survey programs as irradiation at doses resulting from commercial phytosanitation reduces longevity drastically, and that use of radiation induced sterility for SIT may not possibly work on this pest.

Key words: Curculionidae, egg hatch, ionizing radiation, life span, phytosanitation, SIT

INTRODUCTION

Mango pulp weevil, *Sternochetus frigidus* (Fabr.), a quarantined pest in Palawan, has prevented the export of mango (*Mangifera indica* L.) from the Philippines to the USA and other countries with strict quarantine regulations other than those obtained from Guimaras Island, which is certified seed weevil- and pulp weevil-free (Proclamation No. 314, 1993). *S. frigidus* was found in 1987 in the southernmost towns of Bataraza and Brooke's Point in Palawan (Basio et al. 1994), an archipelagic and the largest province in the western Philippines.

The life history, host range, feeding and reproductive behavior were studied by De Jesus & Gabo (2000), De Jesus et al. (2003a, 2003b) and De Jesus (2008). The female lays eggs singly on the fruit peel and covers it with secretions, which hold the eggs in place. Five larval instars develop inside the fruit feeding on the pulp. The fifth instar is voracious and prepares a hole that later becomes a pupal chamber or cell. Total development from egg to adult stage is 32 days at 31^{o}C. The adult remains in the pupal cell inside the fruit until the fruit is fully rotten (De Jesus & Gabo 2000). The adults can live for 1½ years and a female may lay almost 800 eggs in its lifetime with a mean oviposition of 165 days (De Jesus 2008).

The difficulty in identifying *S. frigidus*-infested fruits by visual inspection makes postharvest treatment necessary for disinfestation. Postharvest treatments of mangoes against a closely related species, mango seed weevil, have not been successful using various treatments such as cold, heat or fumigation (Hansen 1991). Irradiation is a viable alternative to disinfest mangoes of *S. frigidus.* It does not leave toxic residues and does not make the food harmful to human health (WHO 1994).

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The use of ionizing radiation as a phytosanitary treatment has been studied for a number of curculionid weevils (Hallman 2016), including *S. frigidus* (Obra et al. 2013, 2014). The mode of action of ionizing radiation as a quarantine treatment involves the breaking of chemical bonds that may prevent the normal development or reproduction of the organism; DNA molecules are the most likely to be affected (Hallman 2003).The physiological and biological effects of radiation were reviewed by Nation & Burditt (1994). Gonads are sensitive to radiation; hence, insects surviving irradiation treatment should be sterile at recommended treatment doses (Tilton & Brower 1983).

The radiation biology of one weevil, *Anthonomus grandis* Boheman, has been extensively studied as a subject for management via the sterile insect technique (SIT) (Hallman 2016). Doses of 100-150 Gy drastically shortened the life span of adults and their competitiveness, resulting in the SIT not being further pursued as a management strategy.

This paper reports on the effects of ionizing radiation on the egg fertility, survival and longevity of adult male and female *S. frigidus* at different doses. This information is useful in two areas: 1) possible use of radiation-induced sterility for SIT, and 2) estimate the risk that adults irradiated at the proper dose for phytosanitary irradiation (PI) purposes will be alive long enough to be detected by pest survey measures.

MATERIALS AND METHODS

Source of Insects

The test insects used in the irradiation studies were obtained from Brooke's Point, Palawan. Due to the absence of artificial diet, rearing of *S. frigidus* was done under field conditions using developing mango as a substrate (Lorenzana & Obra 2013). Pairs of male and gravid female *S. frigidus* were introduced into the bagged mango fruits at least 65 days after flower induction, or approximately at the chicken egg-size fruit stage. The harvesting schedule was based on life history study of De Jesus & Gabo 2000. The infested mangoes with *S. frigidus* adults were securely packed in carton boxes and placed inside crates (five boxes/crate). These were shipped to Manila by plane and were transported to the Philippine Nuclear Research Institute (PNRI) for irradiation studies. All shipments were made possible under a special quarantine permit issued by the Department of Agriculture - Bureau of Plant Industry and were guarded by a Plant Quarantine Officer (Lorenzana & Obra 2016).

Irradiation

Irradiation was carried out at the PNRI Multipurpose Irradiation Facility. The facility has eight individually operated source racks and four turntables. The activity of the Co-60 source was 33.87 kilocuries.

Fricke dosimeters were placed in boxes of mangoes to measure irradiation dose during irradiation. Dosimetry system that was used in the irradiation of mangoes was prepared according to ISO/ASTM Standards and traceable to the National Physical Laboratory of the United Kingdom. Dose mapping was performed first on the mango boxes to determine the positions of the minimum and the maximum dose, the minimum dose rate and the dose uniformity ratio. During routine irradiation, dosimeters were placed outside the mango fruits but at the expected minimum and maximum positions for a four-sided irradiation, i.e., at the front (Plane I), middle (Plane II) and back planes (Plane III) to monitor the dose received by the mangoes Obra et al. (2013).

One- to two week-old adults of *S. frigidus* were irradiated using 25, 50, 75, 100, 150, 300, and 400 Gy. The phytosanitary irradiation dose for *S. frigidus* is currently 165 Gy, but could be as low as 150 Gy (Hallman 2016). Commercial application at a dose uniformity ratio (DUR) of 2.5 (minimum DUR expected when entire pallet loads are irradiated) would result in up to ~400 Gy being absorbed by some of the load when a minimum of 165 Gy is applied. An untreated lot served as the control. The experiment was conducted with three replications per treatment and 100 mangoes per treatment (dose) for all the tests. One hundred mangoes per trial were cut-open at the time of irradiation to serve as check samples.

Post-Irradiation

After conducting irradiation treatments, all test fruits (both treated and control mangoes) still in boxes were held in the laboratory for *S. frigidus* development. Test fruits with adult *S. frigidus* were cut open two days after irradiation or as soon as the mango fruits become partly ripened for ease of removing the weevils. The surviving adult weevils were held in perforated plastic containers and were provided with fresh green mango fruit squares for continued development. About ten days after fruit dissection, separation of sexes of irradiated and untreated adults was done following the procedure of De Jesus et al. (2002).

Adult pairs of ten males and ten females were confined in perforated plastic containers (700 mL capacity; 10.5 cm tall.) for mating. Fifty males and fifty females were used per dose and replication. Mango fruit cubes were provided and replaced daily together with filter paper lining until the time when no more oviposition occurred. Egg hatchability and adult mortality were assessed daily. Survival rates for each dose and in the control weevils were determined.

The same procedure was done on control weevils. *S. frigidus* were held at a room temperature of 27.1 ± 0.9^{o}C and 81.2 ± 7.1% RH and a 12:12 (L:D) h photoperiod.

Data Analysis

Data was analyzed using regression with life data (Minitab Version 14.0).

RESULTS AND DISCUSSION

Longevity refers to the life span of *S. frigidus* while survival rate refers to the probability that *S. frigidus* will live up to a number of days. Non-irradiated adult *S. frigidus* was quite long-lived. In the laboratory, the adult lived a mean of 299 days in males and 228 days in females. Irradiation lowers longevity (Fig. 2) and survival rates of *S. frigidus* (Figure 1a-c).

The higher longevity observed at sub-efficacious doses indicates that male *S. frigidus* were more tolerant to radiation as shown by the regression lines for males and females (Figure 2). The intercepts and slopes were highly significant (p<0.01). The regression lines of males and females were significantly different since there were no overlaps in the 95% confidence intervals of the intercepts and slopes. For the male regression line, the intercept was significantly higher (5.70 compared with 5.34 for the female), and the slope was lower (-0.00525 compared with -0.00397 for the females) (Table 2). This indicates that males were more tolerant than females.

In terms of survival, there were higher survival rates in males than females at 0 to 100 Gy. At 50 Gy, 23% of males lived up to 300 days compared with only 7% for females (Figure 1a). At 150 Gy, only 4% of males and 1% of females lived up to 300 days (Figure 1b). At 300 to 400 Gy, males and females had the same survival rates at 100 days with very few insects survived beyond 200 days (Figure 1c). Measured doses obtained for each target irradiation dose are shown in Table 1. The absorbed dose did not vary considerably from the target doses.

Unlike males, adult females of *S. frigidus* need sources of protein and use some of their energy for egg production. Obra et al. (2013) reported that untreated control female of *S. frigidus* laid a mean of 632 eggs, and adult female treated with 25 and 50 Gy laid about 432, and 46 eggs throughout its lifespan. Increased life span may be a result of decreased fecundity and by lowering the reproductive cost, it allows the insect to live longer.

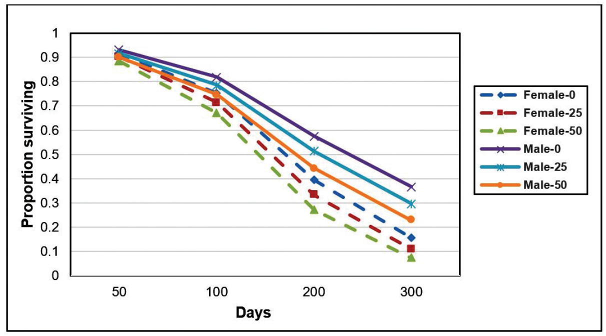

Figure 1a. Survival of adult male and female *S. frigidus* irradiated at 25 and 50 Gy and in the control (untreated).

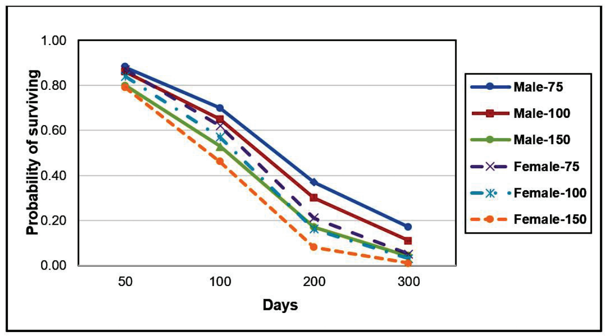

Figure 1b. Survival of adult male and female *S. frigidus* irradiated at 75, 100 and 150 Gy.

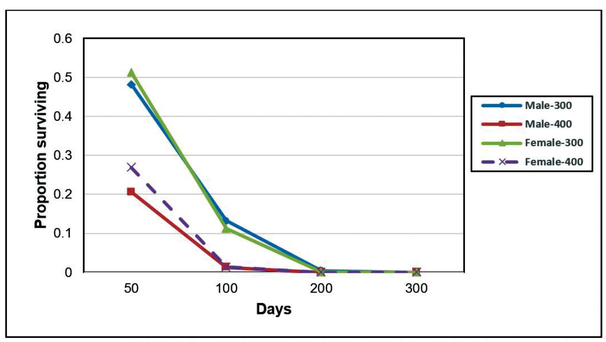

Figure 1c. Survival of adult male and female *S. frigidus* irradiated at 300 and 400 Gy.

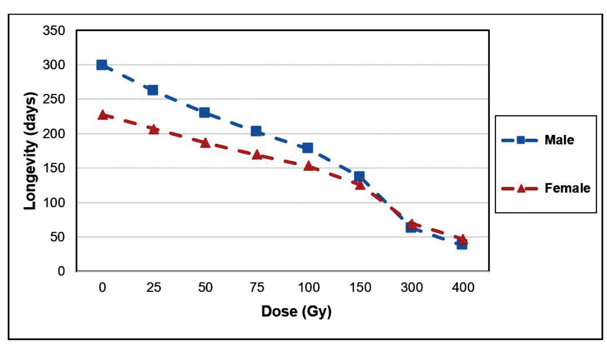

Figure 2. Mean longevity of adult female and male *S. frigidus* vs. dose.

Table 1. Measured doses when adults (*S. frigidus*) were irradiated with doses between 25 and 400 Gy.

Dose (Gy)*	Measured doses (Gy)			
	Avg. min. (±S.E.)	Avg. max. (±S.E.)	Overall min.	Overall max.
25	24.4 ± 1.47	28.4 ± 1.71	28.4	35.3
50	48.2 ± 0.97	54.3 ± 0.77	54.3	58.0
75	72.6 ± 1.28	81.7 ± 1.20	81.7	84.9
100	97.1 ± 1.10	111.2 ± 1.50	109.0	114.4
150	145.1 ± 2.01	163.4 ± 0.95	163.4	168.1
300	282.2 ± 2.67	313.4 ± 2.23	313.8	322.6
400	387.8 ± 0.99	431.4 ± 1.39	431.4	440.4

* Three replications, with 100 fruits per replicate.

Table 2. Comparison of regression equations (on natural log scale) between mean longevity of adult male and female *S. frigidus*.

Sex	Intercept	Slope
Male	5.70** (95% C.I. = 5.65; 5.75)	-0.00525** (95% C.I. = -0.00547; -0.005037)
Female	5.34** (95% C.I. = 5.3; 5.38)	-0.00397** (95% C.I. = -0.00416;-0.0038)

** Highly significant (p<0.01)

The mean number of eggs laid by female *S. frigidus* was 0.2 at 75 Gy and 0 per female at 100 Gy (Obra et al. 2013). At these doses, fecundity was very much reduced as a function dose. No eggs were laid by adult females irradiated with ≥100 Gy (Obra et al. 2013). However, large scale-confirmatory tests using 100 Gy resulted in one adult laying infertile eggs (Obra et al. 2014). At higher doses where fecundity was completely inhibited resulting in complete sterility (Obra et al. 2014) *S. frigidus* females become less radiosensitive than males (Figure 2). This may be a result of reduced nutrient and physiological demands of egg production by the female (Nation & Burditt, 1994).

Egg hatchability was also reduced with increase in dose. Egg hatch was observed only at 25 and 50 Gy and in the control but not at 75 Gy (Table 3). There was a five-fold reduction in hatching rate at 25 Gy and 24-fold at 50 Gy.

Apart from the difficulty of mass-rearing *S. frigidus* in the laboratory for sterile insect releases due to the absence of artificial diet, the results of this study cast doubt of the successful use of SIT based on radiation sterilization of adults against *S. frigidus*, as has been noted with another weevil, *A. grandis* (Hallman 2016). The results also show that the use of irradiation as a phytosanitary treatment against *S. frigidus* using the minimum dose recommended for this insect, 165 Gy, (Obra et al. 2014) results in a lowered risk that adults would be detected by survey programs as irradiation at doses resulting from commercial phytosanitation reduces longevity drastically.

Table 3. Hatching percentage (Mean ± SE) of eggs of *S. frigidus* irradiated as adults.

Dose	Total Eggs*	Egg Hatching (%)(±SE)
0 (Control)	94,848	89.3 ± 0.6
25	64,766	18.3 ± 2.0
50	6,924	3.7 ± 0.6
75	30	0

*Obra et al. 2013

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