Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

Article

Influence of Irradiation on the Biology of the Brown Marmorated Stink Bug (*Halyomorpha halys* Stål)

Hai Nam Nguyen[1], Lloyd Damien Stringer[2] and Ki-Jeong Hong[1]*

[1] Department of Plant Medicine, Sunchon National University, Suncheon 57922, Rep. of Korea; hainguyen309@gmail.com
[2] The New Zealand Institute for Plant and Food Research Ltd, PB 4704, Christchurch 8140, New Zealand; Lloyd.Stringer@plantandfood.co.nz
* Correspondence: curcul@scnu.ac.kr

Abstract: The irradiation biology of the brown marmorated stink bug (BMSB, *Halyomorpha halys* Stål) treated at the nymphal stage was investigated to determine its application for sterile insect technique (SIT). Fifth instar males of BMSB were exposed to gamma-radiation ^{60}Co at different doses of 12, 16, 20, 24 and 64 Gy. Irradiated males were mated with non-irradiated virgin females to assess the longevity of both sexes, female fecundity and fertility of their offspring until the egg stage of the F2 generation. The mortality of each of the developmental stages of the F1 and eggs of the F2 generation was observed to determine whether negative effects from exposure to radiation was inherited. The data indicated that irradiation significantly reduced the lifespan of male insects at doses above 20Gy. Irradiated males did not affect the longevity and fecundity of their female partners, nor either sex of their resulting progeny, but it did reduce the hatch rate of the eggs at all doses tested. The sterility rates of F1 eggs were 55.6%, 73.3%, 74.1% and 74.1% at doses of 12Gy, 16Gy, 20Gy and 24Gy respectively. Eggs were completely sterile (100%) at a dose of 64Gy with no egg hatch recorded. A low hatch rate of F2 eggs illustrated that negative effects from radiation was inherited by the subsequent generation. The results support the potential for the use of SIT for BMSB management by irradiating the fifth instar male nymphs at 16-64Gy.

Keywords: BMSB, *Halyomorpha halys*, inherited sterility, irradiation, SIT.

1. Introduction

The Brown Marmorated Stink Bug, BMSB (*Halyomorpha halys* Stål. 1855, Hemiptera: Pentatomidae), is a polyphagous invasive nuisance insect pest, which is considered native to China, Taiwan, Korea and Japan [1]. With a wide range of host plants [2], its appearance has been reported in the USA (the 1990s), Canada [3], and many countries of Europe such as: Liechtenstein [4], Germany [5], Italy [6], France [7], Hungary [8] and Rusia [9]. Its spread is predicted to continue expanding worldwide [10], and ultimately have the potential to have a wide global range [11]. Aggregation pheromones can be useful for BMSB surveillance [12], and pesticides are frequently used to control this insect pest species [13], but due to environmental issues, sustainable development problems including incompatibility with IPM programs and BMSB's ability to tolerate a range of chemistries [14-16], chemical approaches for its measurement are not desirable.

The sterile insect technique (SIT) is an environmentally-friendly, organic approach, that has proven effective as part of a systems approach to control and eradicate insect pests such as, but not limited to tephritid fruit flies, lepidopteran pest, and mosquitos [17,18]. Some hemipteran species have also been targeted for irradiation [19] but many aspects of the irradiation biology of Hemiptera are poorly developed and to our knowledge, there have been no field applications of hemipteran SIT.

A previous study indicated that irradiating early adult stage BMSB males at 16Gy resulted in 80% of sterility of F1 eggs, which led to a cumulative mortality rate ≈ 99% at the adult stage [20]. Similarly,

© 2020 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

Suckling et al (2019) showed an effect of radiation resulting in lower egg fertility [21]. However, using the same dose rate 16Gy resulted in only 46% sterility, which the authors suggest may have been a result of the irradiation methodology, but could also be due to the age of the virgin male adults at the time of irradiation, which were older than those irradiated in the Welsh et al (2017) paper, as well as using overwintered adults [20,21].

Inherited sterility (IS) in insects was first reported in the mid-1930s [22]. It appeared potentially possible within Hemipteran species after evidence was presented on milkweed bug *Oncopeltus fasciatus* (Dallas) [23] and on *Rhodnius prolixus* (Stål) [24]. The hypothesized mechanism is the persistence of chromosome fragments resulting from irradiation, which is conserved in subsequent untreated generations [23,25,26]. To date, the effect of radiation exposure of the nymphal stage has not been assessed, and inherited sterility biology for BMSB has had some limited investigation.

This work presented here was to determine the impact of radiation on brown marmorated stink bug longevity, fecundity, as well as the inheritance of mortality due to the irradiation of the previous generation to evaluate the feasibility of SIT application for BMSB when irradiating the nymphal stage.

2. Materials and Methods

Insect source: BMSB of all developmental stages were collected using pheromone traps or by hand in the field in Jeollanam-do, Jeollabuk-do, Gyeongsangbuk-do, South Korea. The insects then were reared in the bug rearing room of the Entomology Laboratory, Department of Plant Medicine, Sunchon National University (Suncheon-si, Jeollanam-do, Rep. Korea). Specimens from the subsequent laboratory-reared insects for the experiments.

Experimental design: The experiment was set up with five treatments of five different irradiation doses of 12, 16, 20, 24, 64Gy and control was non-irradiation (0Gy) (n=13-18 males per dose). Male fifth instar nymphs, which were characterized by the presence of a black "U" shape at last abdominal sternite (Figure A1) were exposed to gamma-ray 60Co. Irradiation was implemented at Korea Atomic Energy Research Institute – KAERI (Jeongeup-si, Jeollabuk-do, Rep. Korea)

Pair mating and rearing: Each male from each radiation dose was paired with a non-irradiated virgin female 24 hours after the male adult emerged from the last moult (total pair = 94). Each pair was transferred to a single transparent plastic container 450ml with mesh screen on top for air circulation. Insects were supplied soybean (*Glycine max* (L.) Merr.), groundnut seeds (*Arachis hypogaea* L.), and carrot (*Daucus carota* subsp. *sativus* (Hoffm.) Schübl. & G. Martens) as food [27,28] and a continuous supply of water in a 2ml vial stoppered with cotton. Food was changed every 3 days or at any sign of mold. Small pieces of medical gauze were put into the containers for adults to crawl, settle and deposit their eggs on. All the containers were kept in the incubation chambers with the rearing condition set at 25°C, RH 50-60%, photoperiod of 16:8 hours (light : dark).

Each egg mass, as well as the number of eggs per mass laid by a single pair, was recorded. Fecundity of BMSB was evaluated through the total number of eggs laid by a female. The insects were reared until death to compare the longevity between treatments. Hatching rate of eggs at each treatment was recorded to calculate the sterility due to the exposure to gamma radiation.

F1 nymphs collected in each treatment (if available) were reared through to the adult stage. The number of individuals that survived through each transition was counted. After emerging, the adults of each sex were mated with a non-irradiated conspecific of the opposite sex to investigate any inherited sterility effects occurring from the irradiation of the parental generation. Due to the limitation of F1 nymphs available, F2 egg sterility was only observed for doses 12, 16, 20, 24Gy and control (0Gy) (n=8-15, total pairs=102). Rearing conditions were as described above.

Data analysis: The data were not normally distributed, therefore a non-parametric bootstrap test with pooled resampling method (repetition 10,000 times) was applied. Data were analyzed using R-Studio (ver. 1.2.5019) with the code was modified referring to Dwivedi et al (2017) [29] (see Appendix). The sterility rate then was corrected for control mortality using Abbott's correction (Abbott 1925).

3. Results

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

Male BMSB lifespan varied from 26.9 to 52.0 days depending on the radiation doses, while mean longevity was 66.8 days for the untreated controls. Irradiation had clear impact on the longevity of treated males (p=0.0141, Table 1).

Table 1. Mean (± SD) longevity and fecundity of brown marmorated stink bug *Halyomorpha halys* at various irradiation doses.

Radiation dose	n	Longevity (days)		Fecundity	
		Male[x]	Female	% Female laying egg	Eggs/female
12Gy	15	52.0 ± 40.6ab	44.5 ± 34.7a	46.7	157.3 ± 116.5a
16Gy	13	43.7 ± 37.9abc	44.1 ± 32.1a	69.2	108.3 ± 49.9a
20Gy	14	38.2 ± 31.6bc	37.9 ± 22.9a	42.9	168.0 ± 86.7a
24Gy	18	42.3 ± 22.8bc	46.0 ± 28.9a	66.7	164.8 ± 126.0a
64Gy	18	26.9 ± 24.8c	56.3 ± 48.9a	61.1	168.7 ± 98.1a
0Gy (untreated)	16	66.8 ± 33.4a	39.3 ± 20.6a	75.0	124.8 ± 85.6a
P-value		0.0141	0.6849		0.6535

Values in the same column that are followed by the same letters are not significantly different with 95% level of confidence, [x] identifies the specimens that were irradiated. Rearing condition: 25°C, RH 40-60%, 16:8h L:D.

Longevity of males was significantly reduced at the doses ≥ 20Gy. Among irradiated treatments, the shortest average lifespan recorded was 26.9 days for BMSB irradiated at 64Gy. Data also indicated that treated males did not confer any negative effect to the longevity of their female partners (p=0.6849, Table 1), unlike Welsh et al. (2017) [20].

The percentage of females ovipositing eggs ranged from 43-75% between the treatments, with the control females most likely to lay any eggs (Table 1). Females oviposited on average 108 to 168 eggs in her lifespan, but the difference in the total number of eggs laid by a female between irradiation doses, including control was not statistically significant (p=0.6535, Table 1).

Although the longevity and fecundity of female BMSB was not influenced by their irradiated male partners at all tested doses, hatching rate of eggs laid by those females was significantly different (P<0.05, Table 2). Egg hatch was 67.3% for control females, percentage hatch dramatically reduced to 29.9% at 12Gy, and 17.4 – 18.0% at doses from 16Gy to 24Gy. Sterility rate (Abbott's corrected) was 55.3% at 12Gy and reached around 74% at doses from 16Gy to 24gy. Eggs were completely sterile (100%) at the dose of 64Gy. Sex ratio (male : female) of treatment was greater than control at all tested doses (Table 2)

Table 2. Mean (± SD) hatch rate, sterility rate of egg and adult sex ratio at F1 generation of brown marmorated stink bug *Halyomorpha halys* at different radiation doses.

Radiation dose	No. of egg mass	Total eggs collected	Hatch rate (%)	Sterility rate[1] (%)	Sex ratio (M:F)
12Gy	30	824	29.9 ± 28.7b	55.6	1.60:1
16Gy	34	906	18.0 ± 21.5bc	73.3	1.50:1
20Gy	33	936	17.4 ± 22.2bc	74.1	1.73:1
24Gy	67	1838	17.4 ± 18.0c	74.1	1.36:1
64Gy	39	1056	0	100.0	-
0Gy (untreated)	48	1292	67.3 ± 32.2a		1.15:1
P-value			0.000		

Values in the same column that are followed by the same letters are not significantly different with 95% level of confidence. [1]The sterility rate is Abbott's corrected. Rearing condition: 25°C, RH 40-60% and 16:8h L:D.

The longevity and fecundity of the next generation of BMSB whose parents were directly exposed to radiation are shown in Table 3. Because there were no fertile eggs collected at 64Gy, only four radiation doses remained for both genders at F1. The data illustrates that there was no difference

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

in the longevity of F1 females (p=0.6940, Table 3) and as well as F1 males (p=0.3399, Table 3) from controls. Similarly, longevity and fecundity were not affected by coupling with progeny from irradiated bugs (p=0.6964 and p=0.8991 for males and females, respectively).

Table 3. Mean (± SD) longevity and fecundity of F1 offspring from irradiated brown marmorated stink bugs, *Halyomorpha halys.*

Radiation dose	n	Longevity (days)		Fecundity	
		Male	Female	% female laying egg	Egg/female
12Gy (MxF^{x})	9	55.8 ± 33.6a	34.4 ± 26.3a	33.3	83.0 ± 48.2
16Gy (MxF^{x})	8	61.9 ± 30.9a	38.9 ± 27.5a	37.5	170.7 ± 44.1
20Gy (MxF^{x})	9	43.4 ± 26.3a	35.1 ± 30.7a	33.3	111.5 ± 40.4
24Gy (MxF^{x})	12	59.3 ± 31.8a	50.7 ± 31.6a	58.3	90.4 ± 12.5
0Gy (MxF)	11	49.8 ± 30.3a	40.6 ± 28.6a	54.5	151.5 ± 60.9
P-value		0.6964	0.6940		
12Gy (M^{x}xF)	14	44.6 ± 32.5a	44.1 ± 27.7a	71.4	103.6 ± 71.0a
16Gy (M^{x}xF)	10	36.8 ± 27.4a	52.4 ± 34.4a	70.0	173.6 ± 75.3a
20Gy (M^{x}xF)	15	59.9 ± 26.2a	41.9 ± 34.9a	53.3	153.0 ± 84.1a
24Gy (M^{x}xF)	14	41.1 ± 26.6a	46.6 ± 26.5a	71.4	94.5 ± 75.2a
0Gy (MxF)	11	49.8 ± 30.3a	40.6 ± 28.6a	54.5	151.5 ± 60.9a
P-value		0.3399	0.8991		0.1376

Values in the same column that are followed by the same letters are not significantly different with 95% level of confidence. M-male, F-female; ^{x} indicates the specimens that were progeny of irradiated parents. Due to a very small sample size, post-hoc analysis was not applied for F1 female progeny from irradiated male parents.

There was a limitation in the number of F1 females collected from irradiated male parents and the percentage of females that laid eggs was also low (33.3 to 58.3%), as such, post-hoc tests were not applied. For the males whose fathers were irradiated, the total number of eggs laid by their female partners showed no significant difference (p=0.1376, Table 3). A similar result was observed for females mated to irradiated males.

Hatch rate of F2 eggs was significantly lower for progeny from irradiated males than that for controls. The hatch rate was similar between male and female progeny for all doses except for 24 Gy, where hatch rate of eggs from female F1 progeny was significantly lower than for male F1 (p=0.0271, Table 4).

Table 4. Mean (± SD) hatch rate as well as the number of egg masses and total eggs and sterility rate of F2 generation eggs from irradiated brown marmorated stink bugs, *Halyomorpha halys*.

Radiation dose	Female^{x} x Male				Female x Male^{x}				P value
	No. egg mass	Total egg	Hatch rate (%)	Sterility Rate[1] (%)	No. egg mass	Total egg	Hatch rate (%)	Sterility rate[1] (%)	
12Gy	9	249	17.5 ± 26.7b	70.5	38	1029	33.2 ± 35.6b	44.0	0.1654
16Gy	18	500	20.0 ± 25.0b	66.3	42	1151	22.6 ± 28.5b	61.9	0.7140
20Gy	15	420	16.8 ± 24.8b	71.7	46	1226	29.0 ± 34.7b	51.1	0.1597
24Gy	24	614	8.8 ± 22.8b	85.2	37	1011	25.2 ± 32.1b	57.5	0.0271*
0Gy	38	1013	59.3 ± 35.4a		38	1013	59.3 ± 35.4a		
P-value			0.000				0.000		

Values in the same column that are followed by the same letters are not significantly different with 95% level of confidence. In the same row, * indicates means are significantly different. ^{x} illustrates the individuals that are descended from parents that were directly exposed to radiation. [1]The sterility rate is Abbott's corrected

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

4. Discussion

The sterile insect technique (SIT) is species-specific and has no off-target effects, it has been used successfully for insect eradication on numbers of occasions [18]. However, its background has only been well developed and applied for controlling a small range of pest species of primarily Diptera and Lepidoptera [18,30]. Few studies have been done for investigation of the irradiation biology of Hemiptera including the brown marmorated stink bug [19-21]. More research is still required in order to gain more insight into the irradiation biology of BMSB and Hemiptera more widely, before applying the SIT against BMSB in the field.

Besides choosing the optimized absorbed dose of energy that leads to sterility without losing quality and competitiveness of the insect for SIT programs, the selection of an appropriate developmental stage to expose to radiation is crucially important. For many holometabolous species (having complete metamorphosis), irradiation is often applied at the late pupal stage, or early in the adult stage, when germ tissues have formed and in general, adults are more radio-resistant than pupae, which in turn are more resistant than larvae [17]. In the Pentatomidae, fourth- and fifth-instar nymphs are most frequently selected for irradiation [19]. Recently, there have been some studies on the radiation biology of *H. halys* of different life-stages. Male adult insects within 24h after moult were used for irradiation by Welsh et al (2017) [20], while slightly mature 1-2 week-old virgin and overwintering males of unknown age were investigated by Suckling et al (2019) [21]. Until now, no information has been published on the irradiation biology of a nymphal stage BMSB.

In this study, we also confirmed the reduction in the longevity of irradiated males as observed in Welsh et al (2017) [20]. However, here longevity was only significantly reduced at the doses at and above 20Gy ($p<0.05$, Table 1), while Welsh et al (2017) concluded this occurred at the lower dose 8Gy [20]. The difference might come from radiation sensitiveness of the different life-stages of insects between this trial and the Welsh et al (2017) trial, as similar irradiation and mating methodology was applied in both trials. As the last development stage of nymphs were used for irradiation experiments here, potentially we are observing that newly emerged adults might be more sensitive to radiation than fully mature nymphs. Welsh et al (2017) reported that the longevity of female insects was negatively affected by its irradiated male partner at doses above 32Gy [20]. We did not observe the effect in our study ($p=0.6849$, Table 1). Similarly, Welsh et al (2017) witnessed both the proportion of female depositing eggs and the number of eggs per female tended to be reduced when their male partners were irradiated at doses above 32Gy and no eggs were collected at a dose of 60Gy [20]. But egg-laying activities were still recorded at the dose of 64Gy in our investigation and there was no significant difference in the number of eggs laid by a female between irradiated treatment and control ($p=0.6535$, Table 1).

The sterilization of egg induced by irradiation is thought to be due to the breaking of the chromosome fragmentation of gonial cells, leading to genetically unbalanced gametes and subsequently inhibits the mitosis process, resulting in the death of fertilized eggs or embryos [26,31,32]. Welsh et al (2017) reported that the mortality of F1 egg of BMSB increased in response to irradiation doses absorbed by parental males, and eggs were completely sterile at doses above 32Gy [20]. In our experiment, the dose of 32Gy was not investigated but we observed a similar reduction in the hatching rate of eggs as the dose or radiation increased ($p<0.001$, Table 2). At 16Gy, the sterility rate here reached 73.3% which was slightly lower than Welsh et al (2017) of 80% [20], and much greater than Suckling et al (2019) (45.7%) at the same dose of 16Gy using older adult insects [21]. From 16 to 24Gy, the sterility rate of F1 eggs remained around 73.3-74.1%, which was in contrast to the expectation that the sterility rate should have continued increasing with increasing radiation dose. However, a similar phenomenon was observed from 16-28Gy in Welsh et al (2017) [20]. It appears that the last nymphal stage and newly moulted virgin BMSB are more radiosensitive than older new season virgin and overwintered male BMSB. Age of the same developmental stage also should be taken into consideration, Machi et al. (2019) indicated that *Aedes albopictus* mosquito pupae irradiated at different ages showed differences in the level of their sterility rates [33]. Our nymphs used here

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

took 1-14 days to develop into adults post-irradiation, which may have had an effect on their radio-sensitivity. In addition, different irradiators might generate variable results. In addition, Bakri et al (2005) indicated that practically not all insects within the container receive the same dose while irradiating due to systematic pattern of dose variation [17]. Suckling et al (2019) also thought that the difference in configuration between irradiators might also lead to variable dosimetry outcomes [21]. This information may explain the high standard deviation calculated and variation recorded for longevity (Table 1, Figure A2), fecundity, hatch rate and furthermore the plateau in mortality rate from 16Gy to 24Gy (Figure A3, A4).

Inherited sterility (IS) was first reported in the Soviet Union in the mid-1930s for the silkworm *Bombyx mori* (L.) and in North America for the codling moth *Cydia pomonella* in 1962 [22]. Attributes of IS are that offspring are less fertile than their irradiated parent(s) and there is often a bias towards male offspring in the F1 generation [34]. Within the order Hemiptera, the large milkweed bug *Oncopeltus fasciatus* (Dallas) [23], and *Rhodnius prolixus* (Stål) [24], a reduviid vector of Chagas disease were described as exhibiting inherited sterility. The mechanism of this was said to be from the production of broken chromosomes and translocation of fragments induced by irradiation [23,25,26]. The new chromosomal formation was reported translocating in the sperm of milkweed bug *Oncopeltus fasciatus* and persist for three generations [23]. Beta chromosomes were found to be maintained for several generations in *Rhodnius prolixus* (Hemiptera: Reduviidae) [24]. Stringer et al (2017) also reported the formation of beta chromosomes in *Nezara viridula* L., which have some potential for transgenerational mortality [26].

Unlike the paternal generation, the longevity of F1 BMSB did not show any response to irradiation doses. No significant difference in the lifespan was found in either the male or female progeny. Because of the small number of F1 female offspring surviving and low proportion of females laying eggs (number of female ovipositing eggs ~ 3-7, Table 3), there was limited opportunity to evaluate the fecundity of the F1 female offspring. For the male progeny from irradiated male parents, it was observed that they did not affect the longevity of their female partners ($p=0.1376$, Table 3), similar to their male parent. Low hatching rate of eggs at all irradiation doses ($p<0.001$, Table 4) indicated that the effect of radiation treatment at the paternal generation had been passed on to their progeny of both sexes. The influence of irradiation on the F1generation was not the same for males and females, with F2 egg hatch rate between the male and female F1 BMSB progeny differing at 24Gy. The sterility rate of F2 eggs laid by female progeny was greater than those oviposited by the parental generation. LaChance (1985) also stated that F1 male and female offspring in some Lepidopteran species are more sterile than the irradiated parental (P1) generation, and there is a bias towards F1 male progeny than female progeny being produced [34]. There were slightly fewer F1 females produced than males in this study (Table 2), but more work needs to be done with a larger sample size to clarify whether male bias exists in irradiated BMSB.

In order to achieve an effective SIT control program, the selection of optimum irradiation doses to get the balance between sterility and competitiveness is of prime concern and this is driven by factors including ratio of wild and release treated population [35]. A 74% sterility rate of F1 at 16Gy is quite modest data for an eradication program, however, it does not only not affect the insect's lifespan but also accumulate to 97.6% mortality rate at F1 adult (Figure 4A), reduces the fertility of eggs at the F2 generation. So the dose of 16Gy can be applied at the initial stage of an incursion when wild BMSB population is relatively high. The release of fully sterile insects at 64Gy is recommended later when wild population density is not as great. However, due to the reduction in longevity induced by the higher doses that may lead to lower competitiveness, increasing the release frequency of the treated population may need to be noted. There are still questions regarding the competitiveness status of those irradiated BMSB at the nymphal stage, and the overflooding ratio of treated and wild BMSB required for population suppression. Further, we reiterate Suckling et al (2019), in that work also needs to consider uniform irradiation dose with low variance [21] as well as the optimal physiological state of the insect.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

5. Conclusions

In summary, irradiation reduced the longevity of treated male insects but did not affect the longevity nor fecundity of their female partners at both the parental and subsequent F1 generation. However, it induced reduced fertility of F1 and F2 egg stage suggesting the potential for inheritance of sterility contributing significant information for sterile insect technique. For SIT, mass rearing of insects, as well as selection of the optimum irradiation threshold, play a crucially important role. Normally, the insects should be irradiated at the early stage when the sperm cells are not well developed to get high effectiveness [17]. In the case of BMSB which is an incomplete metamorphic insect species, our results suggest that the SIT can be applied for BMSB management when irradiating nymphal stage insects at doses from 16-64Gy, complementary to previous research conducted by Welsh et al (2017) and Suckling et al (2019) [20,21].

Author Contributions: Conceptualization, K-J.H.; methodology, K-J.H., H.N.N; data curation, H.N.N; formal analysis, H.N.N.; investigation, H.N.N; writing—original draft preparation, H.N.N.; writing—review and editing, H.N.N., L.D.S., and K-J.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The New Zealand Institute for Plant and Food Research Limited.

Acknowledgments: We would like to express our sincere thanks to the Korea Atomic Energy Research Institute (Jeongeup-si, Jeollabuk-do, Rep. Korea) for irradiation of insect samples and the Better Border Biosecurity (www.b3nz.org) collaboration. The authors are also thankful to the Department of Plant Medicine, Sunchon National University, Korea for facilities support while implementing this work.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

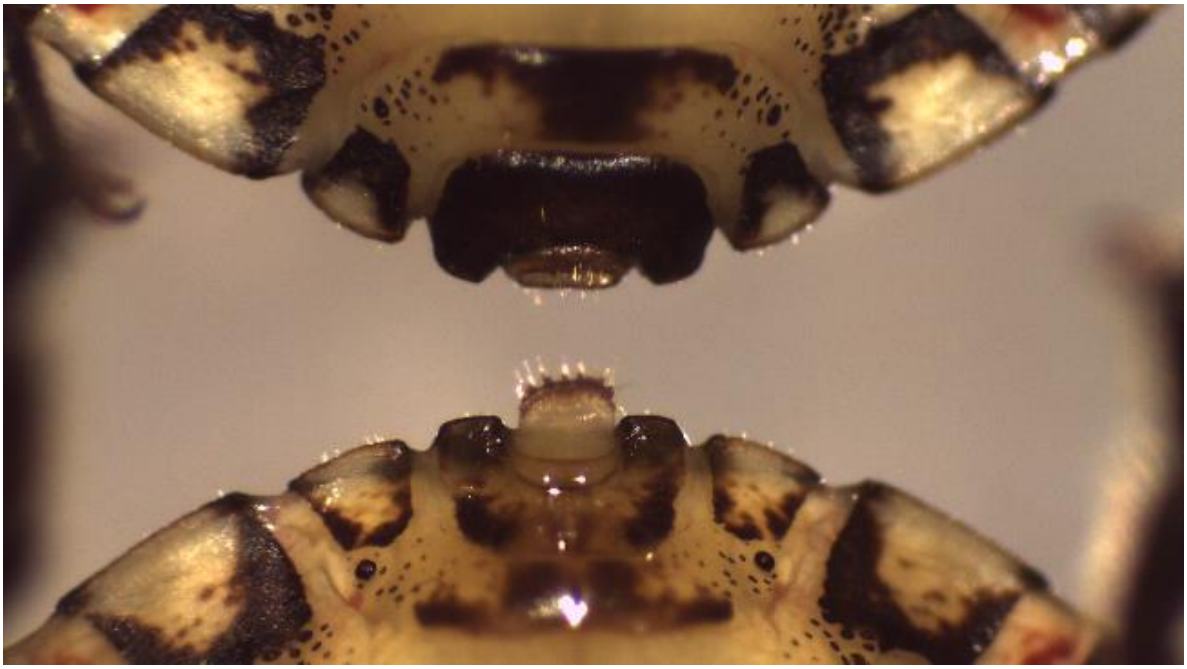

Figure A1. Male *Halyomorpha halys* (upper) at the last nymphal stage exhibits a black "U" shape at last abdominal sternite. A female *H. halys* is shown for comparison (lower).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

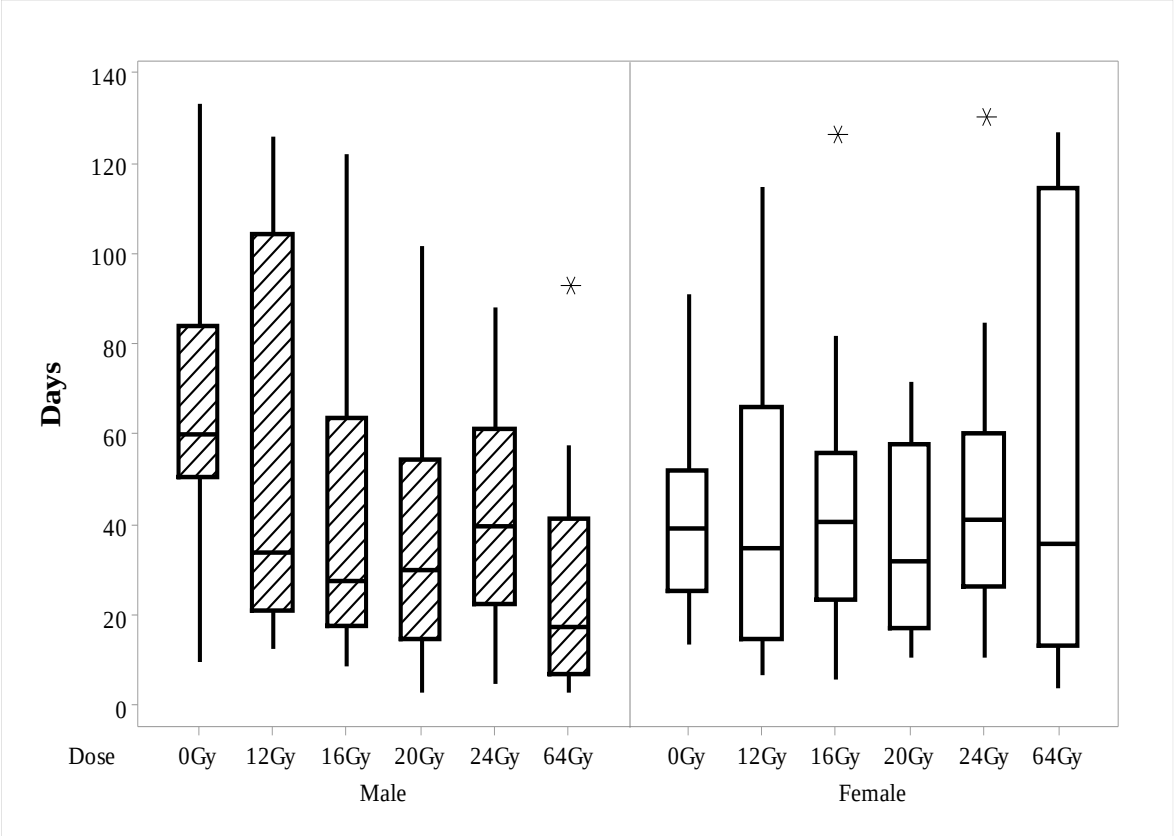

Figure A2. Quartiles boxplot of male and female longevity of brown marmorated stink bug *Halyomorpha halys* (parental generation) versus irradiation dose. The upper line extends to the highest value, in contrast with lower line which determines the lowest value; the box within indicates 25 and 75% interquartile range; the horizontal line shows median; (*) symbol indicates outlier values which in this case is the maximum or minimum value.

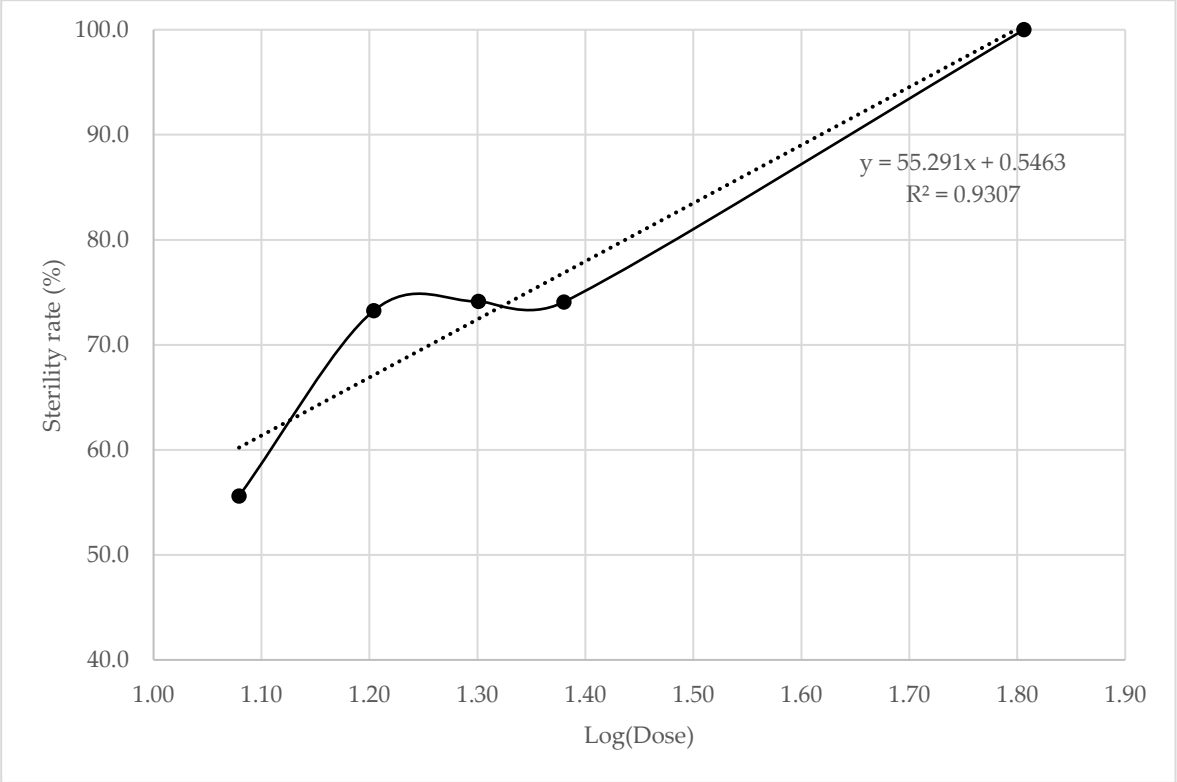

Figure A3. Relationship of sterility rate (y) of F1 egg of *Halyomorpha halys* and irradiation dose (x) was described by y = 55.291.log(x) + 05463; R^2 = 0.9307.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

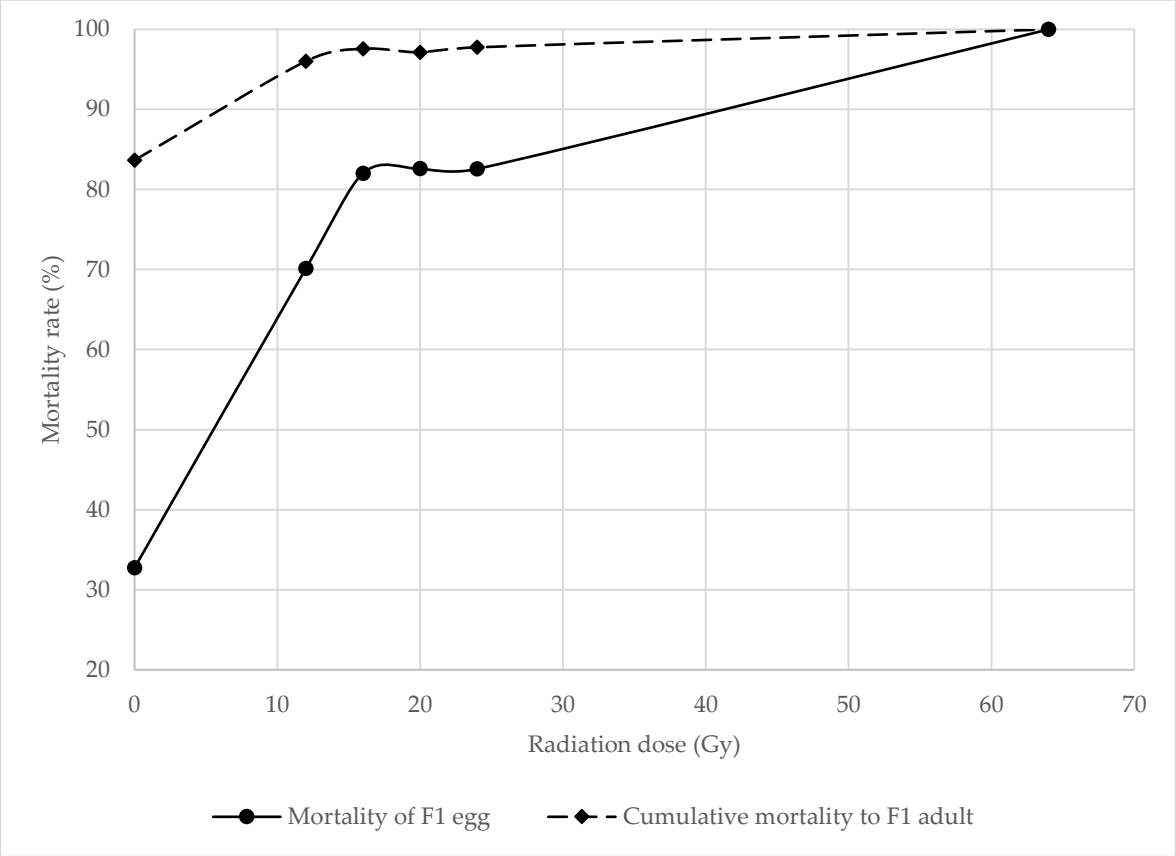

Figure A4. The cumulative mortality rate of brown marmorated stink bug *Halyomorpha halys* from F1 egg to the emergence of adults at different radiation doses.

Appendix B

R code for bootstrap function.

```
# Nonparametric bootstrap t-test for two samples comparison
bootR = 10000
bootstrap = function(x1, x2, bootR){
  t.values = numeric(bootR)
  for (j in 1:bootR) {
    set.seed(j)
    group1 = sample(c(x1, x2), size = length(x1), replace = T)
    group2 = sample(c(x1, x2), size = length(x2), replace = T)
    if (sd(group1)==0 & sd(group2)==0) {t.values[j] = NA}
    else {t.values[j] = t.test(group1, group2, paired = F)$statistic}
  }
  p.boot = mean(abs(t.values) >= abs(t.test(x1, x2 ,paired = F)$statistic))
  p.boot
}
bootstrap(x1, x2, bootR)
# Nonparametric bootstrap f-test for multi-comparison
bootR = 10000
bootstrapx = function(x, y, bootR){
  f.values = numeric(bootR)
  for (j in 1:bootR) {
    set.seed(j)
    x1 <- sample(x, size = sum(y=="0Gy"), replace = T)
    x2 <- sample(x, size = sum(y=="12Gy"), replace = T)
```

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1


```
    x3 <- sample(x, size = sum(y=="16Gy"), replace = T)
    x4 <- sample(x, size = sum(y=="20Gy"), replace = T)
    x5 <- sample(x, size = sum(y=="24Gy"), replace = T)
    x6 <- sample(x, size = sum(y=="64Gy"), replace = T)
    rex = c(x1,x2,x3,x4,x5,x6)
    if(sd(x1)==0 & sd(x2)==0 & sd(x3)==0 & sd(x4)==0 & sd(x5)==0 & sd(x6)==0){f.values[j] = NA}
    else {f.values[j] = na.omit(anova(lm(rex~y))$"F value")}
  }
  p.boot = mean(abs(f.values) >= na.omit(anova(lm(x~y))$"F value"))
  p.boot
}
bootstrapx(x, y ,bootR)
```

References

1. Hoebeke, E.R.; Carter, M.E. *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. *Proceedings of the Entomological Society of Washington* **2003**, *105*, 225-237.
2. CABI. *Halyomorpha halys* (brown marmorated stink bug). Availabe online: https://www.cabi.org/cpc/datasheet/27377 (accessed on 25 Nov 2019).
3. Fogain, R.; Graff, S. First records of the invasive pest, *Halyomorpha halys* (Hemiptera: Pentatomidae), in Ontario and Quebec. *Journal of the Entomological Society of Ontario* **2011**, *142*, 45-48.
4. Arnold, K. *Halyomorpha halys* (Stål, 1855), a stink bug species newly detected among the European fauna (Insecta: Heteroptera, Pentatomidae, Pentatominae, Cappaeini). *Mitteilungen Thuringer Entomol* **2009**, *16*, 19.
5. Heckmann, R. First evidence of *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) in Germany. *Heteropteron* **2012**, *36*, 17-18.
6. Pansa, M.; Asteggiano, L.; Costamagna, C.; Vittone, G.; Tavella, L. First discovery of *Halyomorpha halys* in peach orchards in Piedmont. *Informatore Agrario* **2013**, *69*, 60-61.
7. Callot, H.; Brua, C. *Halyomorpha halys* (Stål, 1855), the marmorated stink bug, new species for the fauna of France (Heteroptera: Pentatomidae). *L'Entomologiste* **2013**, *69*, 69-71.
8. Vetek, G.; Papp, V.; Haltrich, A.; Redei, D. First record of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Heteroptera: Pentatomidae), in Hungary, with description of the genitalia of both sexes. *Zootaxa* **2014**, *3780*, 194-200, doi:10.11646/zootaxa.3780.1.8.
9. Gapon, D.A. First records of the brown marmorated stink bug Halyomorpha halys (Stål, 1855) (Heteroptera, Pentatomidae) in Russia, Abkhazia, and Georgia. *Entomological Review* **2016**, *96*, 1086-1088, doi:10.1134/s001387381608011x.
10. Zhu, G.; Bu, W.; Gao, Y.; Liu, G. Potential geographic distribution of brown marmorated stink bug invasion (*Halyomorpha halys*). *PLoS One* **2012**, *7*, e31246, doi:10.1371/journal.pone.0031246.
11. Kriticos, D.J.; Kean, J.M.; Phillips, C.B.; Senay, S.D.; Acosta, H.; Haye, T. The potential global distribution of the brown marmorated stink bug, *Halyomorpha halys*, a critical threat to plant biosecurity. *Journal of Pest Science* **2017**, *90*, 1033-1043, doi:10.1007/s10340-017-0869-5.
12. Khrimian, A.; Zhang, A.; Weber, D.C.; Ho, H.Y.; Aldrich, J.R.; Vermillion, K.E.; Siegler, M.A.; Shirali, S.; Guzman, F.; Leskey, T.C. Discovery of the aggregation pheromone of the brown marmorated stink bug (*Halyomorpha halys*) through the creation of stereoisomeric libraries of 1-bisabolen-3-ols. *J Nat Prod* **2014**, *77*, 1708-1717, doi:10.1021/np5003753.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

13. Rice, K.B.; Bergh, C.J.; Bergmann, E.J.; Biddinger, D.J.; Dieckhoff, C.; Dively, G.; Fraser, H.; Gariepy, T.; Hamilton, G.; Haye, T., et al. Biology, Ecology, and Management of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae). *Journal of Integrated Pest Management* **2014**, *5*, A1-A13, doi:10.1603/ipm14002.
14. Kuhar, T.P.; Kamminga, K. Review of the chemical control research on *Halyomorpha halys* in the USA. *Journal of Pest Science* **2017**, *90*, 1021-1031, doi:10.1007/s10340-017-0859-7.
15. Lee, D.H.; Wright, S.E.; Leskey, T.C. Impact of insecticide residue exposure on the invasive pest, *Halyomorpha halys* (Hemiptera: Pentatomidae): analysis of adult mobility. *J Econ Entomol* **2013**, *106*, 150-158, doi:10.1603/ec12265.
16. Leskey, T.C.; Lee, D.H.; Short, B.D.; Wright, S.E. Impact of insecticides on the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae): analysis of insecticide lethality. *J Econ Entomol* **2012**, *105*, 1726-1735, doi:10.1603/ec12096.
17. Bakri, A.; Mehta, K.; Lance, D.R. Sterilizing Insects with Ionizing Radiation. In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, Dyck, V.A., Hendrichs, J., Robinson, A.S., Eds. Springer Netherlands: Dordrecht, 2005; 10.1007/1-4020-4051-2_9pp. 233-268.
18. Suckling, D.M.; Stringer, L.D.; Stephens, A.E.; Woods, B.; Williams, D.G.; Baker, G.; El-Sayed, A.M. From integrated pest management to integrated pest eradication: technologies and future needs. *Pest Manag Sci* **2014**, *70*, 179-189, doi:10.1002/ps.3670.
19. IDIDAS. International Database on Insect Disinfestation and Sterilization. Availabe online: https://nucleus.iaea.org/sites/naipc/ididas/Pages/Browse-IDIDAS.aspx (accessed on 25 Oct 2019).
20. Welsh, T.J.; Stringer, L.D.; Caldwell, R.; Carpenter, J.E.; Suckling, D.M. Irradiation biology of male brown marmorated stink bugs: is there scope for the sterile insect technique? *Int J Radiat Biol* **2017**, *93*, 1357-1363, doi:10.1080/09553002.2017.1388547.
21. Suckling, D.M.; Cristofaro, M.; Roselli, G.; Levy, M.C.; Cemmi, A.; Mazzoni, V.; Stringer, L.D.; Zeni, V.; Ioriatti, C.; Anfora, G. The Competitive Mating of Irradiated Brown Marmorated Stink Bugs, *Halyomorpha halys*, for the Sterile Insect Technique. *Insects* **2019**, *10*, doi:10.3390/insects10110411.
22. Carpenter, J.E.; Bloem, S.; Marec, F. Inherited Sterility in Insects. In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, Dyck, V.A., Hendrichs, J., Robinson, A.S., Eds. Springer Netherlands: Dordrecht, 2005; 10.1007/1-4020-4051-2_5pp. 115-146.
23. LaChance, L.E.; Degrugillier, M. Chromosomal Fragments Transmitted through Three Generations in Oncopeltus (Hemiptera). *Science* **1969**, *166*, 235-236, doi:10.1126/science.166.3902.235.
24. Maudlin, I. The inheritance of radiation induced semi-sterility in *Rhodnius prolixus*. *Chromosoma* **1976**, *58*, 285-306, doi:10.1007/bf00292095.
25. LaChance, L.E.; Degrugillier, M.; Leverich, A.P. Cytogenetics of inherited partial sterility in three generations of the large milkweed bug as related to holokinetic chromosomes. *Chromosoma* **1970**, *29*, 20-41, doi:10.1007/bf01183659.
26. Stringer, L.D.; Harland, D.P.; Grant, J.E.; Laban, J.; Suckling, D.M. Effect of 40Gy irradiation on the ultrastructure, biochemistry, morphology and cytology during spermatogenesis in the southern green stink bug *Nezara viridula* (Hemiptera: Pentatomidae). *bioRxiv* **2017**, 10.1101/171991, 171991, doi:10.1101/171991.
27. Dingha, B.N.; Jackai, L.E.N. Laboratory rearing of the brown marmorated stink bug (Hemiptera: Pentatomidae) and the impact of single and combination of food substrates on development and survival. *The Canadian Entomologist* **2017**, *149*, 104-117, doi:10.4039/tce.2016.39.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 February 2020 doi:10.20944/preprints202002.0255.v1

28. Funayama, K. A new rearing method using carrots as food for the brown-marmorated stink bug, *Halyomorpha halys* (Heteroptera: Pentatomidae). *Applied Entomology and Zoology* **2006**, *41*, 415-418, doi:10.1303/aez.2006.415.
29. Dwivedi, A.K.; Mallawaarachchi, I.; Alvarado, L.A. Analysis of small sample size studies using nonparametric bootstrap test with pooled resampling method. *Stat Med* **2017**, *36*, 2187-2205, doi:10.1002/sim.7263.
30. Klassen, W.; Curtis, C.F. History of the Sterile Insect Technique. In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, Dyck, V.A., Hendrichs, J., Robinson, A.S., Eds. Springer Netherlands: Dordrecht, 2005; 10.1007/1-4020-4051-2_1pp. 3-36.
31. Lance, D.R.; McInnis, D.O. Biological Basis of the Sterile Insect Technique. In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, Dyck, V.A., Hendrichs, J., Robinson, A.S., Eds. Springer Netherlands: Dordrecht, 2005; 10.1007/1-4020-4051-2_3pp. 69-94.
32. Robinson, A.S. Genetic Basis of the Sterile Insect Technique. In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, Dyck, V.A., Hendrichs, J., Robinson, A.S., Eds. Springer Netherlands: Dordrecht, 2005; 10.1007/1-4020-4051-2_4pp. 95-114.
33. Ricardo Machi, A.; Rodrigues Mayne, R.; Adriani Gava, M.; Bergamin Arthur, P.; Arthur, V. Gamma Radiation Sterilization Dose of Adult Males in Asian Tiger Mosquito Pupae. *Insects* **2019**, *10*, doi:10.3390/insects10040101.
34. LaChance, L.E. *Genetic methods for the control of Lepidopteran species : status and potential*; United States Dept. of Agriculture, Agricultural Research Service ;: [Washington, D.C.] :, 1985; Vol. ARS-28.
35. Parker, A.; Mehta, K. Sterile Insect Technique: A Model for Dose Optimization for Improved Sterile Insect Quality. *Florida Entomologist* **2007**, *90*, 88-95, 88.