Influence of Irradiation on the Biology of the Brown Marmorated Stink Bug (Hemiptera: Pentatomidae)

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Abstract

Fifth-instar brown marmorated stink bug (Halyomorpha halys Stål) nymphs were treated by gamma-radiation 60Co at different doses of 8–64 Gy to investigate their irradiation biology and potential for the sterile insect technique (SIT). At adult emergence, males were mated with non-irradiated virgin females to assess the longevity of both sexes, female fecundity, and egg sterility. Biological parameters of their F1 progeny were investigated to determine whether negative effects from parental exposure to radiation were inherited. Results showed that irradiation significantly reduced the lifespan of male insects at doses above 20 Gy. Irradiated males did not affect the longevity and fecundity of their female partners, nor of their resulting adult progenies, but it did reduce the developmental duration of nymphs as well as weight gain of male F1 offspring. Egg hatch was significantly reduced at all tested doses and reached complete sterility at 64 Gy. Low hatch of eggs produced by F1 or F1 crossed adults indicated that negative effects from radiation were inherited by the subsequent generation. But F1 male offspring were not less fertile than their irradiated male parent, unlike what was observed in Lepidoptera. The results support the potential for the use of SIT for H. halys management by irradiating the fifth-instar male nymphs at doses from 16 Gy to 64 Gy.

Graphical Abstract

Key words: fecundity, inherited sterility, longevity, fifth-instar nymph, sterile insect technique

The brown marmorated stink bug (Halyomorpha halys Stål. 1855, Hemiptera: Pentatomidae) is a polyphagous invasive nuisance insect pest, which is considered native to China, Taiwan, Korea, and Japan (Hoebike and Carter 2003). With a wide range of host plants (CABI 2019), its appearance has been reported in the United States, Canada (Fogain and Graff 2011), and many countries of Europe such as Liechtenstein (Arnold 2009), Germany (Heckmann 2012), Italy (Pansa et al. 2013), France (Callot and Brua 2013), Hungary (Vétek et al. 2014), and Russia (Gapon 2016). It is predicted to continue expanding worldwide (Zhu et al. 2012) until a wide global range is
realized (Kriticos et al. 2017). Aggregation pheromones can be useful for H. halys surveillance (Leskey et al. 2012d, Khrimian et al. 2014, Rice et al. 2014, Suckling et al. 2019b), and pesticides are frequently used to control this pest. Unfortunately, many pesticides do not show high levels of effectiveness, while the remaining available materials effective against H. halys are generally broad spectrum (Leskey et al. 2012a, 2012b; Lee et al. 2013; Khu and Kamminga 2017; Leskey and Nielsen 2018) that could interfere with IPM practices and subsequently led to the secondary pest outbreaks (Leskey et al. 2012c). Thus, chemical approaches for its control are not always desirable.

The sterile insect technique (SIT) is an environmentally friendly approach that has proven effective as part of a systems approach to eradicate insect pests such as, but not limited to tephritid fruit flies, lepidopteran pests, and mosquitoes (Bakri et al. 2005, Klassen and Curtis 2005, Suckling et al. 2014). Some hemipteran species have also been targeted for irradiation (IDIDAS 2019), but many aspects of the irradiation biology of Hemiptera (true bugs) are poorly developed and to our knowledge, there have been no large-scale applications of hemipteran SIT in the field.

The influence of irradiation on fertility was investigated previously for adult H. halys. Irradiating early adult-stage H. halys males at 16 Gy resulted in 80% egg sterility, which led to a cumulative mortality of 99% at the F1 adult stage (Welsh et al. 2017). Suckling et al. (2019a) applied the same dose but showed a lower sterility of only 46% when using 1- to 2-wk-old and overwintered males for irradiation. However, the authors suggested that results may have been confounded by the irradiation methodology used, whereby the insect could move towards or away from the source. Up to now, no information about the effect of radiation exposure of the H. halys nymphal stage has been assessed.

Inherited sterility (IS) in insects was first reported in the mid-1930s (Carpenter et al. 2005). It appeared to be possible within Hemipteran species after evidence was presented on milkweed bug Oncopeltus fasciatus (Dallas) (Hemiptera: Lygaedae) (LaChance and Degrugillier 1969) and on Rhodnius prolixus (Stål) (Hemiptera: Reduviidae) (Maudlin 1976). The hypothesized mechanism involved the persistence of chromosome fragments resulting from irradiation, which is conserved in subsequent untreated generations (LaChance and Degrugillier 1969, LaChance et al. 1970, Stringer et al. 2017, preprint). To date, inherited sterility biology for H. halys has been only limited investigation.

This present study aimed at determining the impact of radiation on the longevity, fecundity, and fertility of brown marmorated stink bug, as well as the inherited sterility due to the irradiation of parental generation to evaluate the feasibility of SIT application for H. halys when irradiated at the nymphal stage.

Materials and Methods

Insect Source

Brown marmorated stink bug of all developmental stages were collected using pheromone traps or by hand in the field in Jeollanam-do, Jeollabuk-do, Gyeongsangbuk-do in South Korea. The adult insects were isolated and reared in the bug rearing cages size of 40- x 40- x 40-cm under room condition (T = 26 ± 2°C, RH: 50–60%) at the Entomology Laboratory, Department of Plant Medicine, Sunchon National University (SCNU, Suncheon-si, Jeollanam-do, Republic of Korea). A light system was set up to ensure the insects were exposed to a photoperiod of 16:8 h (L: D). Soybean [Glycine max (L.) Merr. (Fabales: Fabaceae)], groundnut seeds (Arachis hypogaea L. (Fabales: Fabaceae)), and carrot [Daucus carota subsp. sativus (Hoffm.) Schüb. & G. Martens (Apiales: Apiaceae)] were supplied as food (Funayama 2006, Dingha and Jackai 2017), and a water-soaked cotton in 50-ml cup was used for water supply. Pots of green beans (Phaseolus vulgaris L. (Fabales: Fabaceae)) were put inside the cages for resting and oviposition. Eggs were collected twice a week and kept in Petri dishes for hatching. After molting to the second instar, nymphs were transferred to top-mesh-screen plastic rectangle boxes of 15 x 20 x 10 cm at a density of 50 bugs per box and allowed to develop to the fifth instar. Food and water were provided as above. Male fifth-instar nymphs, which were characterized by the presence of a black ‘U’ shape at last abdominal sternite (Fig. 1), were selected for irradiation.

Irradiation

The insects were irradiated at Korea Atomic Energy Research Institute (KAERI, Jeongeup-si, Jeollabuk-do, Republic of Korea). The male nymphs were isolated, randomly selected, and kept in each plastic canister for radiation exposure. Canisters were placed in the center of the irradiation chamber (Cobalt-60 gamma irradiator IR-222, Nordion Inc., Ottawa, Ontario, Canada), and then exposed to gamma-ray at different dose rates of 8, 12, 16, 20, 24, 34, and 64 Gy/h for 1 h. Control insects remained in the laboratory. The absorbed doses were measured by Alanine Pellet Dosimeters (Bruker Instruments, Rheinstetten, Germany). The actual absorbed doses in irradiated samples differed by less than 10% from the respective target doses. Irradiated insects were reared through to adults in an incubator (Sanyo Incubator—MIR253, Sanyo Electric Co. Ltd.) set at 25°C, RH 50–60%, and photoperiod of 16:8 h (L: D). Food supply and rearing methods were as described above for nymphs. The fifth-instar nymphs in this study took 1–14 d to develop into adults after irradiation.

Experimental Design

In 2019, the experiment on fifth-instar nymphs was conducted with radiation doses of 12, 16, 20, 24, and 64 Gy. Males from irradiated cohorts were placed with a non-irradiated virgin female of the same age within 24 h after emergence. The control treatment comprised non-irradiated (0 Gy) pairs. Each mating pair was transferred to a single labeled transparent plastic container with a volume of 450 ml and a mesh screen on top for air circulation. The experiment was replicated 13–18 times for each dose. Insects were supplied soybean, groundnut seeds, and carrot as described above and a continuous

![Fig. 1. 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).](image-url)
supply of water in a 2-ml vial stoppered with cotton. Food was changed every 3 d 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. All the containers were kept in the incubator with the rearing condition set at 25°C, RH 50–60%, and photoperiod of 16:8 h (L: D).

Egg masses and the number of eggs per mass laid by each pair were counted and transferred to a single Petri dish (d = 3.5 cm), labeled, and dated. Any damage of eggs due to cannibalistic behavior or eggs laid separately was not considered for the egg hatch calculation. The date and number of nymphs hatched from each egg mass from treatments were recorded to determine the hatchability and the sterility induced by gamma radiation. The fecundity of brown marmorated stink bug was evaluated by the total number of eggs from a female that laid eggs. The insects were reared until death to assess the longevity in each treatment. F1 nymphs hatched from eggs of each treatment above (if available) were allowed to aggregate and molt to second instars, then were transferred to rearing boxes (as above) for subsequent molts. The rearing method and food supply were as above for the nymphs. At emergence, the number of F1 adults was counted and sexed. The nymphal mortality of the F1 generation was estimated by dividing the number of nymphs that successfully developed to adults by the number of first instars hatched. Adult progeny of both sexes were continuously mated with a non-irradiated conspecific of the opposite sex to investigate any inherited sterility effects occurring from the irradiation of the parental generation. There were no nymphs collected from 64 Gy, and the sterility of eggs produced by F1 adults was estimated for doses 12, 16, 20, and 24 Gy and control (0 Gy). Each mating pair was replicated 8–15 times with a total of 102 pairs crossed. Experiment procedure, food supply, and rearing conditions were as described above for parental generation.

In 2020, the fifth-instar nymphs were irradiated at 8, 16, 24, and 32 Gy, plus a control (0 Gy), resulting in 26–30 adult males that were mated with non-irradiated females of the same age. Adult longevity, female fecundity, and egg hatch were evaluated as in 2019. At hatching, nymphs were allowed to aggregate and molt to second instar, and then were transferred to a single-insect breeding dishes (d = 5.5 cm) for individual rearing to evaluate developmental duration and mortality of each molting transition. Because there was a limited amount of progeny surviving at higher doses, we followed 72–100 nymphs for doses of 0, 8, and 16 Gy only. Breeding dishes were kept in the incubator set at 25°C, RH 50–60%, and photoperiod of 16:8 h (L: D). Nymphs were checked daily, and the date of each molting and death was recorded. The number of nymphs that developed into adults was counted and sexed. Weight gain of adults (both genders) was measured using a digital scale Scout Pro Model: SPG402F (Ohaus Corporation, Parsippany, NJ, USA). The F1 adults of both sexes were continuously paired with a non-irradiated specimen of the opposite sex to investigate inherited effects (8–20 pairs). The first 20 egg masses for radiation dose and 30 egg masses from control pairs were collected to assess F2 egg hatch and sterility as done in 2019.

### Results

Irradiation had a clear impact on the longevity of irradiated males in both 2019 and 2020 (P = 0.0141 [Table 1] and P = 0.0001 [Table 2]). There was a reduction in male longevity with increasing radiation dose, with the significance observed at doses ≥ 20 Gy (2019) and ≥ 24 Gy (2020) (Tables 1 and 2). Data also indicated that the relationship between male longevity and the maturity of fifth-instar nymph at the time of irradiation was statistically significant (P < 0.001). The negative correlation (r = −0.42) between the time taken to final molt and subsequent adult longevity indicated that when fifth-instar nymphs were irradiated at an earlier stage, their longevity at adults tended to decrease. By contrast, adult longevity showed an increasing trend when nymphs were treated close to final molt (Fig. 2).

Irradiated males did not confer any negative effect to the longevity of their female partners (P = 0.6849; Table 1). The percentage of non-irradiated females mated with irradiated males ovipositing

### Table 1. Mean (±SE) longevity and fecundity of fifth-instar-irradiated male brown marmorated stink bug *Halyomorpha halys* and percentage sterility of eggs (experiment in 2019)

| Radiation dose (Gy) | n  | Ir-male     | Female     | Female that laid eggs (%) | Eggs/female | No. eggs mass observed | Hatchability (%) | Sterility (%) |
|---------------------|----|-------------|------------|---------------------------|-------------|------------------------|-----------------|--------------|
| 0 Gy (untreated)    | 16 | 66.8 ± 4.4a | 39.3 ± 5.1a| 75.0a                     | 157.3 ± 44.1a| 48                     | 67.3 ± 4.6a     | –            |
| 12 Gy               | 15 | 52.0 ± 10.5ab| 44.5 ± 9.0a| 46.7a                     | 108.3 ± 16.6a| 30                     | 29.9 ± 5.3b     | 55.6         |
| 16 Gy               | 13 | 43.7 ± 10.5abc| 44.1 ± 8.9a| 69.2a                     | 168.0 ± 35.5a| 34                     | 18.0 ± 3.7bc    | 73.3         |
| 20 Gy               | 14 | 38.2 ± 8.4bc | 37.9 ± 6.1a| 42.9a                     | 164.8 ± 36.4a| 33                     | 17.4 ± 3.8bc    | 74.1         |
| 24 Gy               | 18 | 42.3 ± 5.4bc | 46.0 ± 6.8a| 66.7a                     | 168.7 ± 29.6a| 67                     | 17.4 ± 2.1c     | 74.1         |
| 64 Gy               | 18 | 26.9 ± 5.8c  | 56.3 ± 11.5a| 61.1a                     | 124.8 ± 24.7a| 39                     | 0              | 100.0        |

Values in the same column that are followed by the same letters are not significantly different with 95% level of confidence. ‘Ir-’ indicates the specimens that were irradiated.
eggs did not display any difference among the treatments in 2019 ($P = 0.3970$; Table 1) but significantly reduced at 32 Gy in 2020 experiment ($P = 0.0100$; Table 2). In 2019, females oviposited on average 108–168 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). The repeated experiment in 2020 with doses from 8 to 32 Gy also showed same results (Table 2). Although the females appeared to live longer and laid more eggs in the control, no difference was found between treatments ($P = 0.0712$ and $P = 0.1968$ for longevity and fecundity, respectively).

The hatchability of eggs laid by females among treatments was significantly different ($P < 0.0001$ in both Tables 1 and 2). Significant reductions in egg hatch were observed from the lowest dose of 8 Gy and continued to decline until no eggs hatched at a dose of 64 Gy. The relationship between sterility and radiation dose was highly correlated, with about 89.5% variation in sterility explained by a regression model ($P < 0.001$; Fig. 3).

Irradiation of the parental generation did not affect the developmental time of their own eggs ($P = 0.4409$), but there was a significant reduction in the duration of the combined F1 nymphal stages. The time from first instar to adult was 2 (at 8 Gy) and 8 d (at 16 Gy) faster than in the control. Results also indicated that weight gain of male progenies from irradiated fathers was significantly lower than that of the control. However, there was no difference in the weight of female F1 adults. More males were produced than females at all treatments (Table 3; Fig. 4).

The longevity and fecundity of the F1 generation of H. halys from males directly exposed to radiation are shown in Table 4. Unlike the directly treated parent generation, no significant differences were observed in comparison with control longevity ($P = 0.6940$ and $P = 0.3399$, respectively). Similarly, their non-treated partners’ longevity was also not affected ($P = 0.6964$ and $P = 0.8991$ for males and females, respectively). There was a limitation in the number of F1 female offspring collected from irradiated male parents. The percentage of females that laid eggs was very low (33.3–58.3%), as such, post-hoc

### Table 2. Mean (±SE) longevity and fecundity of fifth-instar-irradiated male brown marmorated stink bug Halyomorpha halys and percentage sterility of eggs (experiment in 2020)

| Radiation dose  | n  | Ir-male | Female | Female that laid egg (%) | Eggs/female | No. eggs mass observed | Hatchability (%) | Sterility (%) |
|-----------------|----|---------|--------|-------------------------|-------------|-----------------------|-----------------|--------------|
| 0 Gy (untreated)| 30 | 71.9 ± 3.9a | 64.0 ± 4.2a | 90.0a | 219.9 ± 17.7a | 240 | 67.8 ± 1.9a | – |
| 8 Gy            | 30 | 62.4 ± 4.0ab | 51.2 ± 4.2a | 70.0ab | 187.7 ± 21.7a | 154 | 36.8 ± 2.0b | 45.6 |
| 16 Gy           | 27 | 61.4 ± 5.6ab | 51.6 ± 4.8a | 74.1ab | 170.4 ± 17.5a | 132 | 18.2 ± 1.5c | 74.2 |
| 24 Gy           | 26 | 53.2 ± 4.8bc | 48.8 ± 4.7a | 84.6a | 168.5 ± 18.3a | 153 | 9.3 ± 0.8d | 86.3 |
| 32 Gy           | 29 | 39.2 ± 4.1c | 48.5 ± 4.0a | 51.7b | 163.0 ± 22.2a | 101 | 2.9 ± 0.4e | 95.8 |
| $P$-value       | <0.0001 | 0.0712 | 0.0100 | 0.1968 | – | <0.0001 |

Values in the same column that are followed by the same letters are not significantly different with 95% level of confidence. ‘Ir-’ indicates the specimens that were irradiated.

![Fig. 2. Correlation between male longevity and fifth-instar nymph maturity of Halyomorpha halys irradiated at dose above 16 Gy. The relationship between male longevity ($y$) and nymph maturity at irradiation ($x$) can be described by a linear model: $y = 60.069 - 2.9672x$ ($P < 0.001$, $R^2 = 0.1745$). The dot line shows the predicted male longevity for any days to adult value.](image-url)
tests were not applied for female fecundity. For the F1 male offspring, their female partners’ fecundity showed no significant difference ($P = 0.1580$), a similar result as observed in their parental generation.

The hatchability of eggs produced by F1 adults was significantly lower for progeny from irradiated males than for controls ($P < 0.0001$; Tables 4 and 5). The hatchability was similar between male and female progeny for all doses except for 24 Gy, where egg hatch from female F1 progeny was significantly lower than for male F1 adults ($P = 0.0271$; Table 4).

**Discussion**

The SIT is species-specific and has no off-target effects. It has been used successfully for the eradication of various insect pest species (Suckling et al. 2014). In general, insects are often irradiated at the stage when germ tissues have formed. In the Pentatomidae, fourth- and fifth-instar nymphs are most frequently selected for irradiation (Bakri et al. 2005, IDIDAS 2019). Recently, there have been some studies on the radiation biology of *H. halys* of different life stages. Previously, male adult insects (within 24 h of emergence) were used for irradiation (Welsh et al. 2017), while slightly mature 1-2-wk-old virgin and overwintering males of unknown age have been investigated (Suckling et al. 2019a). Until now, no information has been published on the irradiation biology of the nymphal stage of *H. halys* as well as the biology of its subsequent generations.

In this study, we confirmed the reduction in the longevity of irradiated males as observed in Welsh et al. (2017). However, here

![Fig. 3. Relationship between sterility ($y$) of F1 egg of *Halyomorpha halys* and irradiation dose ($x$) was described by $y = 64.768 \log(x) - 8.9188$ ($P < 0.0001$, $R^2 = 0.8948$). Diamond shapes indicate sterility obtained from the 2020 experiment (doses of 8, 16, 24, and 32 Gy); Circle dots were from 2019 (doses of 12, 16, 20, 24, and 64 Gy).](image)

**Table 3.** Mean developmental time (days ± SE) of immature stage, survival of nymphs, adult weight and sex ratio of F1 offspring *Halyomorpha halys* produced from fifth-instar-irradiated male parents (experiment in 2020)

| Radiation dose | n  | Eggs | 1st instar | 2nd instar | 3rd instar | 4th instar | 5th instar | Nymphal stage | Survival (%) | Weight gain (g) | Sex ratio (M: F) |
|----------------|----|------|------------|------------|------------|------------|------------|---------------|--------------|----------------|-----------------|
| 0 Gy           | 100| 5.06 | 4.14 ±     | 6.85 ±     | 6.39 ±     | 7.60 ±     | 10.17 ±    | 33.00 ±       | 95.0| 70.5| 71.6| 62.3| 0.091a| 0.132a| 1.14:1 |
|                |    | 0.05 | 0.07b      | 0.20a      | 0.18a      | 0.46a      | 0.31a      | 0.59a         | 8 Gy         | 4.98 | 4.29 ± | 6.66 ± | 6.22 ± | 6.95 ± | 9.46 ± | 31.42 ± | 94.0| 69.1| 66.2| 60.5| 0.079b| 0.123a| 1.16:1 |
|                |    | 0.02 | 0.06a      | 0.17a      | 0.20a      | 0.38a      | 0.24ab     | 0.42b        | 16 Gy        | 5.00 | 4.63 ± | 5.79 ± | 6.23 ± | 6.59 ± | 8.94 ± | 25.76 ± | 83.3| 65.0| 59.0| 65.2| 0.077b| 0.122a| 1.25:1 |
|                |    | 0.07 | 0.07b      | 0.12b      | 0.26a      | 0.23a      | 0.26b      | 0.50b        | P-value      | 0.4409 | < 0.0001 | 0.0021 | 0.8791 | 0.2173 | 0.0175 | 0.0042 | 0.0373 | 0.4898 |

Values in the same column that are followed by the same letters are not significantly different with 95% level of confidence.
longevity was only significantly reduced at doses and above 20 Gy ($P < 0.05$; Table 1), whereas the above-mentioned authors observed this to occur from 8 Gy. It was reported that the longevity of female insects was negatively affected by their irradiated male partners at doses above 32 Gy (Welsh et al. 2017). We did not observe this effect in experiments from both years. Similarly, the previous study showed both the percentage of female depositing eggs and the number of eggs per female tended to be reduced when their male partners were irradiated at doses above 32 Gy and no eggs were oviposited at a dose of 60 Gy (Welsh et al. 2017). The reduction in the percentage of females that laid eggs was found at 32 Gy in 2020. However, egg-laying activities were still recorded at the doses of 32 and 64 Gy in our investigation, and there was no significant difference in the number of eggs laid by a female between irradiated treatment and control ($P = 0.6535$ in Table 1 and $P = 0.1986$ Table 2).

Welsh et al. (2017) reported that the mortality of F1 eggs from irradiated $H$. halys increased in response to irradiation doses absorbed by parental males, and eggs were completely sterile at doses above 32 Gy.
et al. 2005). It appeared to be possible within Hemipteran species when the insects absorbed the same amount of radiation. (Diptera: Culicidae) (Ernawan et al. 2017), than lower dose rate results between studies (Hall 1972, Bakri et al. 2005). A higher dose irradiation dose rate differences could lead to variations in reproductive response with multiple tools, 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 the ratio of wild to released treated insects (Parker and Mehta 2007). This study provided a reliable model for determining the irradiation dose to achieve a desired sterility level for a SIT program. A 74% sterility of F1 at 16 Gy is quite modest for an eradication program; however, it does not only not affect the insect’s fitness but also accumulates to 97.6% mortality at F1 adult (Fig. 4), thus reduces the fertility of eggs produced by the F1 generation. A sterilizing dose of 16 Gy can be applied at the initial stage of an incursion when wild H. halys population is relatively high. The release of fully sterile insects at 64 Gy (or nearly fully sterile at 32 Gy) is recommended later when the wild population density is not as great but tolerance of progeny is low. However, due to the reduction in longevity induced by the higher doses that may lead to lower competitiveness, an increase in the release frequency and/or the over-flooding ratio of the irradiated insect population may be needed.

While there are still questions around sufficient rearing or collection of H. halys and its compatibility with other management techniques, our results and previous authors’ results indicate that the SIT against H. halys is technically feasible. What is required next are larger trials in field cages or open field that validate or otherwise the laboratory results.

Table 5. The sterility of *Halyomorpha halys* eggs produced by F1 offspring of the fifth-instar-irradiated male parents (experiment in 2020)

| Pair type (female: male) | Egg sterility | Hatchability (%) | Sterility (%) |
|---------------------------|---------------|------------------|---------------|
| N: N                      | 30            | 72.27 ± 5.16a    | –             |
| 8 Gy: N                   | 20            | 23.99 ± 4.51b    | 66.8          |
| 16 Gy: N                  | 20            | 9.03 ± 2.01c     | 87.5          |
| P-value                   | <0.0001       |                  |               |
| N: N                      | 30            | 72.27 ± 5.16a    | –             |
| N: 8 Gy                   | 20            | 27.95 ± 6.22b    | 61.3          |
| N: 16 Gy                  | 20            | 14.18 ± 3.07b    | 80.4          |
| P-value                   | <0.0001       |                  |               |

Values in the same column that are followed by the same letters are not significantly different with 95% level of confidence; the upper half shows pairs in which females were the progeny of irradiated parents and the lower shows pairs in which males were the progeny of irradiated parents; N: non-irradiated specimens.

We also observed a similar reduction in the hatchability of eggs as the dose of radiation increased (**P** < 0.0001; Tables 1 and 2). However, there was still a small number of nymphs hatched from 32 Gy eggs in our study. At 16 Gy, the sterility reached 73.3–74.1%, which was slightly lower than that reported in Welsh et al. (2017: i.e., 80%), and much greater than that reported in Suckling et al. (2019a: i.e., 45.7%), at the same dose of 16 Gy when they used older adult insects.

The difference with previous studies might come from radiation sensitiveness of the different life stages of insects used between trials. As the last development stage of nymphs were used for irradiation experiments here, potentially newly emerged adults might be more radio-sensitive than fully mature nymphs and older non-overwintered virgin and overwintered male *H. halys*. Age of the same developmental stage also should be taken into consideration. For example, research on the mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae) indicated that mosquito pupae irradiated at different ages showed differences in their sterility level (Machi et al. 2019). Our nymphs used here took 1–14 d to develop into adults post-irradiation. We anticipate this is due to the mixed age of the fifth-instar nymphs at the time of irradiation. Longevity estimates suggest that the younger nymphs were more sensitive to radiation than the last stage nymphs, as we observed that the longevity of the arising male adults was lower than what would be expected from a simple arithmetic reduction of the number of days post-irradiation from the adult longevity estimate (Fig. 2). In addition, not all insects in the container overwintered and survived the same radiation dose while irradiating due to a systematic pattern of dose variation (Bakri et al. 2005). Suckling et al. (2019a) thought that the configuration difference between irradiators might also lead to variable dosimetry outcomes. The irradiation dose rate differences could lead to variations in results between studies (Hall 1972, Bakri et al. 2005). A higher dose rate resulted in higher mortality in coding moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) (Burditt et al. 1989), while it led to shorter longevity in male mosquitoes *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) (Ernawan et al. 2017), than lower dose rate when the insects absorbed the same amount of radiation.

IS was first reported in the Soviet Union in the mid-1930s for the silkworm *Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae) and in North America for the coding moth *C. pomonella* in 1962 (Carpenter et al. 2005). It appeared to be possible within Hemipteran species (LaChance and Degrugillier 1969, Maidlin 1976). For *H. halys* here, the low hatchability of eggs from F1 adults at all sterilizing doses (**P** < 0.0001; Tables 4 and 5) indicated that the effect of radiation treatment at the paternal generation had been passed on to their progeny of both sexes. However, the F1 adults did not appear to be more sterile than their irradiated male parents, unlike in some Lepidopteran species where offspring are more sterile than the irradiated parental (P0) generation (Carpenter et al. 2005). Further, there is a bias towards F1 male progeny than female progeny being produced (Curtis et al. 1973, LaChance 1985).

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Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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Bakri, A., K. Mehta, and D. R. Lance. 2005. Sterilizing insects with ionizing radiation, pp. 233–268. In V. A. Dyck, J. Hendrichs, and A. S. Robinson (LaChance and Degrugillier 1969, Maidlin 1976). For *H. halys* here, the low hatchability of eggs from F1 adults at all sterilizing doses (**P** < 0.0001; Tables 4 and 5) indicated that the effect of radiation treatment at the paternal generation had been passed on to their progeny of both sexes. However, the F1 adults did not appear to be more sterile than their irradiated male parents, unlike in some Lepidopteran species where offspring are more sterile than the irradiated parental (P0) generation (Carpenter et al. 2005). Further, there is a bias towards F1 male progeny than female progeny being produced (Curtis et al. 1973, LaChance 1985). There were slightly fewer F1 females produced than males in this study, but more genetic work needed to be done to clarify whether male bias exists in irradiated *H. halys* or higher mortality of female nymphs occurs prior to adults.

To achieve an effective SIT control program as part of a wider response with multiple tools, 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 the ratio of wild to released treated insects (Parker and Mehta 2007). This study provided a reliable model for determining the irradiation dose to achieve a desired sterility level for a SIT program. A 74% sterility of F1 at 16 Gy is quite modest for an eradication program; however, it does not only not affect the insect’s fitness but also accumulates to 97.6% mortality at F1 adult (Fig. 4), thus reduces the fertility of eggs produced by the F1 generation. A sterilizing dose of 16 Gy can be applied at the initial stage of an incursion when wild *H. halys* population is relatively high. The release of fully sterile insects at 64 Gy (or nearly fully sterile at 32 Gy) is recommended later when the wild population density is not as great but tolerance of progeny is low. However, due to the reduction in longevity induced by the higher doses that may lead to lower competitiveness, an increase in the release frequency and/or the over-flooding ratio of the irradiated insect population may be needed.

While there are still questions around sufficient rearing or collection of *H. halys* and its compatibility with other management techniques, our results and previous authors’ results indicate that the SIT against *H. halys* is technically feasible. What is required next are larger trials in field cages or open field that validate or otherwise the laboratory results.
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