Norikuni Kumano[1], Dai Haraguchi[2], Kaori Tsurui-Sato[3]

Effects of X-ray irradiation on male sperm transfer ability and fertility in the sweetpotato weevils *Euscepes postfasciatus* (Coleoptera: Curculionidae) and *Cylas formicarius* (Coleoptera: Brentidae)

[1] Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

[2] Okinawa Prefectural Agricultural Research Center, Itoman, Okinawa 901-0336, Japan

[3] Center for Strategic Research Projects, University of the Ryukyus; Senbaru, Nishihara, Okinawa 903-0213, Japan

Corresponding author: Norikuni Kumano; E-mail address: nrkumano@obihiro.ac.jp; Tel: +81-155-49-5490

Abstract

Gamma radiation from isotopic sources has been used in sterile insect technique (SIT) programs worldwide, but it might be difficult to continue using these sources in future SIT programs because of social issues. Therefore, an alternative sterilization source to gamma rays, such as X-rays, needs to be developed. The physical properties of radiation are different between gamma rays and X-rays; for example, X-rays have a shorter penetration depth than gamma rays. Therefore, X-rays may not fully confer male sterility, depending on the target pest insects. The present study investigated whether the West-Indian sweetpotato weevil *Euscepes postfasciatus* (Fairmaire) and the sweetpotato weevil *Cylas formicarius* (Fabricius) are sterilized by X-rays generated in a low-energy X-ray irradiator, without deterioration of male mating ability, at the doses currently used in the eradication programs for *E. postfasciatus* (150 Gy) and *C.*

formicarius (200 Gy) using gamma rays at Okinawa, Japan. Results demonstrated that it is possible to use X-rays in future SIT programs for *E. postfasciatus* and *C. formicarius*, because X-ray irradiated males were almost sterilized without deterioration of their mating ability.

Keywords

Sterile insect technique・*Ipomoea batatas*・area-wide integrated pest management (AW-IPM)・alternative sterilization source

Introduction

The sterile insect technique (SIT) consists of target pest insect species mass production and sterilization, followed by area-wide release into the field. The released sterile males compete with wild males for females of the same species, and these, when mated with sterile males, lay sterile eggs thereby reducing wild populations of the target pest (Knipling 1955). The application of SIT is a biological, environmentally friendly, effective, and sustainable method within integrated pest management approaches (Dyck et al. 2005).

Insect sterilization is indispensable in SIT programs. Historically, gamma radiation from isotopic sources such as Cobalt 60 (^{60}Co) or Cesium 137 (^{137}Cs) was frequently used in pest control programs including SIT (Bakri et al. 2005; FAO/IAEA 2007). Although radioisotopes are advantageous for safely sterilizing large numbers of mass-reared target pest insects compared to chemosterilants (Bakri et al. 2005; FAO/IAEA 2007), these sources need to be regularly replenished (Bakri et al. 2005). In addition, their transboundary transportation is becoming difficult because of potential social issues, including terrorism (FAO/IAEA 2007; Mastrangelo et al. 2010). Hence, the development of an alternative sterilization source is required in future SIT programs (FAO/IAEA 2007; Mastrangelo et al. 2010).

The use of X-rays is considered a suitable alternative to radioisotopes to induce insect sterility because X-rays have many advantages over radioisotope irradiators (Bakri et al. 2005; United States Food and Drug Administration 2004). For example, because X-rays are electrically powered, much less shielding is required at irradiation facilities, and national legislation requirements are simpler, and transportation costs are lower than those of radioisotopes (FAO/IAEA 2007). A low-energy, self-contained X-ray irradiator for SIT has already been developed (FAO/IAEA 2007; Mastrangelo et al. 2010; Mehta and Parker 2011), and it was recently introduced in Thailand to sterilize the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (FAO/IAEA 2017).

The physical properties of gamma rays are known to differ from those of X-rays. Gamma rays emitted by the atomic nucleus have very short wavelengths (less than 10 pm), but X-rays consist of various, relatively long wavelengths, ranging from 1 pm to 10 nm (FAO/IAEA 2007, 2008; Mastrangelo et al. 2010). Because the energy of photons is generally inversely proportional to wavelength, X-rays are usually considered lower-energy electromagnetic radiation than gamma rays. Therefore, differences in physical properties, such as the penetration depth between gamma rays and X-rays, would affect the effectiveness of insect sterilization.

Studies on insect irradiation using X-rays are needed for future SIT applications (Bakri et al. 2005; FAO/IAEA 2007, 2008; Mastrangelo et al. 2010).

In Japan, four agricultural pest species, namely the melon fly *Bactrocera cucurbitae* (Coquillett), the tephritid fruit fly *Bactrocera latifrons* (Hendel), the West-Indian sweetpotato weevil *Euscepes postfasciatus* (Fairmaire), and the sweetpotato weevil *Cylas formicarius* (Fabricius), are currently released after sterilization by gamma rays emitted by ^{60}Co in AW-IPM programs in Okinawa and Kagoshima Prefectures (Hayashikawa 2005; Kuba et al. 2003; Miyaji et al. 2000; Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries 2013). Among these, the weevils *E. postfasciatus* and *C. formicarius* are important pests of sweet potato, *Ipomoea batatas* (L.) Lam., in the South Pacific, the Caribbean, some parts of Central South America, and in the Southern Islands of Japan (Chalfant et al. 1990; Jansson and Raman

1991; Raman and Alleyne 1991; Yasuda 1993; Yasuda and Kohama 1990). Eradication programs for these weevils include the irradiation of adult *E. postfasciatus* and *C. formicarius* after emergence at a dose of 150 and 200 Gy ^{60}Co gamma rays, respectively, to fully achieve sterilization (Kuba et al. 2003). Using X-rays generated by a high-power electron linear accelerator with 5 MeV have already been confirmed to sterilize these weevils at a dose of 150 Gy (Follett 2006). However, the practical use of a high-power electron linear accelerator in SIT programs is not easy considering initial and running costs and the size of the irradiator.

Sterilization procedures have negative impacts not only on reproductive cells, but also on somatic cells, and it is well known that irradiation deteriorates sterile insects' sexual competitiveness and longevity, as damage and dose rates have a trade-off relationship (e.g., Bakri et al. 2005; Calkins and Parker 2005; Lance and McInnis 2005; Sakurai 2000; Sakurai et al. 1994, 2000a,b). Gamma irradiation damages the midgut epithelial tissue of *E. postfasciatus* and *C. formicarius* (Sakurai, 2000; Sakurai et al. 1994, 2000a,b). Recent studies reported that the X-rays generated by the low-energy self-contained X-ray irradiator are sufficient for SIT of dipteran species such as *Ceratitis capitata* (Wiedemann), *Anastrepha fraterculus* (Wiedemann), and *Aedes albopictus* (Skuse) (e.g., Mastrangelo et al. 2010; Yamada et al. 2014). However, research on sterilization using X-rays for application in SIT is extremely rare in coleopteran species, with the exception of a few cases (e.g., Downey et al. 2015; Follett 2006). Because, unlike dipteran pupae, the bodies of sweetpotato weevil species are protected by a thick exoskeleton (Sherman and Tamashiro, 1954), they might not be sterilized by low energy X-rays at the currently used dose, as indicated by previous studies (Follett 2006; Kumano et al. 2008a,b, 2010a,b; Sakurai 2000; Sakurai et al. 1994; 2000a,b). However, sterilization efficiency by low-energy X-rays is yet to be tested in sweetpotato weevil species. Therefore, the present study examined male *E. postfasciatus* and *C. formicarius* mating ability and fertility after irradiation using the commercial X-ray irradiator for future SIT programs. Considering the eradication programs for these species in Japan, *E. postfasciatus* and *C. formicarius* male weevils were irradiated at 125 to 200 Gy and 125 to 250 Gy, respectively. Because some studies have shown that irradiation sensitivity varies among the genotypes of some species including coleopterans (e.g., Fisher 1997, Hallman 2003), we used mass-reared strains of both weevils as the tested cultures.

Materials and methods

General methods

All weevils used in the experiments were reared at Okinawa Prefectural Agricultural Research Center (OPARC) facilities in Itoman, Okinawa, Japan. Irradiation of sweetpotato weevils was conducted at the Tropical Biosphere Research Center, University of the Ryukyus (TBRCUR), Nishihara, Okinawa, Japan. Experiments were conducted in the laboratory at OPARC from June to August 2016 for *E. postfasciatus* and from August to November 2016 for *C. formicarius*. Because experimental methods differed between the two weevil species, they are described separately.

Tested weevils

The *E. postfasciatus* stock culture strain used in the experiments was obtained from about 1000 adult weevils collected at Yaese Town, Okinawa, Japan (26°7′N, 127°42′E), in August 2012.

Weevils were reared on sweet-potato roots and kept in the laboratory at the OPARC for 22 generations under the following conditions: 25 ± 1 °C; light (L):dark (D) regime of L14:D10 (light between 0400 and 1800 h); and relative humidity (RH) of 50–90%. This stock culture was maintained with more than 2000 weevils every generation. Sweet-potato roots were dissected in June 2, 2016 (about six weeks after inoculation with weevils), and pupae were extracted from pupal chambers to obtain virgin weevils. The extracted pupae were transferred to plastic Petri dishes (Falcon, Corning, NY, USA; diameter, 100 mm; height, 15 mm), where they were maintained until emergence. Newly emerged weevils were collected and sexed under a stereomicroscope according to the previously described sexing method (Kohama and Sugiyama 2000). These newly emerged and sexed adults were considered to be 0-day-old and each sex was maintained in separate plastic mesh cups (volume, 250 ml; diameter, 8 cm; height, 5 cm) containing sweet-potato roots (about 50 g) until irradiation or experimentation.

The *C. formicarius* stock culture strain was obtained from about 1500 adult weevils collected at Itoman, Okinawa, Japan (26°11′N, 127°70′E), in August 2006. These weevils were reared under the same conditions as *E. postfasciatus* for 64 generations at the OPARC, and the stock culture was also maintained with more than 2000 weevils every generation. Sweet-potato roots were dissected in October 19, 2016 (about five to six weeks after inoculation) to obtain virgin weevils. The extracted *C. formicarius* pupae were treated in the same manner as *E. postfasciatus* pupae until emergence, and newly emerged adult weevils were sexed based on the morphological characteristics of their antenna (Sherman and Tamashiro 1954). These newly collected adults were considered to be 0-day-old, and each sex was maintained as indicated for *E. postfasciatus* adults until irradiation or experimentation.

Weevil irradiation with X-rays

General methods: The X-rays used were generated by a low-energy self-contained X-ray irradiator designed for medical and industrial purposes (MBR-1505R-2, Hitachi, Tokyo, Japan, Fig. 1a,b) held at TBRCUR. Two replicate groups were used for each weevil species (on June 20 and 24, 2016 for *E. postfasciatus* and on November 1 and 2, 2016 for *C. formicarius*).

Euscepes postfasciatus: The age of male weevils at irradiation was nine to 14 days. For each replicate group, 30 male weevils were irradiated per X-ray dose (0, 125, 150, 175, and 200 Gy).

Cylas formicarius: The age of male weevils at irradiation was 13 to 14 days. For each replicate group, 30 male weevils were irradiated at each X-ray dose (0, 100, 150, 200 and 250 Gy). On the day of each irradiation for both species, the plastic mesh cups (90 ml) containing male weevils within a cooling container (about 20 °C) were transported from the OPARC to the TBRCUR. At the laboratory of TBRCUR, weevils were transferred to small plastic Petri dishes (Falcon; diameter, 40 mm; height, 15 mm) by every ten males 2 h before irradiation. During each irradiation, three Petri dishes containing male weevils were arranged in a concentric circle on the turntable of the irradiator (circle diameter was about 55 cm and the distance between the irradiation source and Petri dishes was 30 cm, Fig. 1b, 2a,b). The turntable was rotated (7.2 rpm) during the X-ray irradiation to achieve irradiation uniformity. All irradiations were conducted at 150 kV and 5 mA using this method. Photon energy (E) was calculated as follows:

$$E = \frac{hc}{\lambda}$$

where h is the Planck constant, c is the speed of light in vacuum, and λ is the photon wavelength. Therefore, the theoretical value of the minimum X-ray wavelength based on the tube voltage (150 kV) used in the present study is 0.0083 nm. Because not all energy is converted to photon energy due to the characteristics of the X-ray irradiator, the irradiation wavelengths used in the present study were equal to or longer than 0.0083 nm. In this irradiator, cumulative irradiation dose is monitored by a dosimeter probe set alongside the plastic Petri dishes (Fig. 2a,b) at every irradiation, and the X-ray is automatically shut off when the cumulative irradiation dose exceeds the setting dose. All irradiations were conducted at 26 ± 1 °C, and the range of dose rates was between 6.25 and 7.63 Gy/min. After irradiation, treated male weevils were packed into plastic cups (90 ml), transported to the laboratory of the OPARC on a cool package, and maintained at 25 ± 1 °C; L14:D10 (light from 0400 h) until the pairing.

Effects of irradiation on male mating ability and fertility

Irradiated males were allowed to mate with non-irradiated virgin adult females (*E. postfasciatus*: 10- to 19-day-old; *C. formicarius*: 13- or 14-day-old). In the mating ability test, we randomly paired one treated male and one virgin female and placed them in one plastic cup (90 ml) with a piece of sweet-potato root (about 20 g). This test started at 1630 h on the day after pairing males and females, and *E. postfasciatus* and *C. formicarius* pairs were kept for ten and seven days, respectively. Sixty pairs were established for each species and irradiation treatment. After the mating/inoculation period, we removed weevils and dissected females, in water and using fine forceps, under a binocular microscope to verify the presence of sperm in their spermatheca. Removed spermathecae were placed on a slide with a drop of 0.9% saline and a cover glass (18 × 18 mm). Samples were observed under a polarizing microscope (Eclipse E600, Nikon, Tokyo, Japan) at 160× magnification. The presence of sperm in the spermatheca was used to calculate the proportion of inseminated females in each irradiation treatment. The inoculated sweet-potato roots were kept in separate plastic cups under 25 ± 1 °C, L14:D10, and 50–90% RH for about 46 days. The roots were then dissected, and larvae, pupae, and newly emerged weevils were counted.

Statistical analyses

Fisher’s exact test was used to compare the frequency of dead weevils during the mating/inoculation period between sexes. Generalized linear mixed models (GLMMs) were used to test sperm transfer ability and fertility (Bolker et al., 2009). In the GLMM analyses, the presence of sperm in female spermatheca (binomial error with a logit link function) and the number of total progeny including immature individuals (Poisson error with a log link function) were used as response variables, and irradiation dose and replicates were used as the fixed and random effects, respectively. Generalized linear models (GLMs) were used to test male survival during the mating/inoculation period. In this analysis, male survival (binomial error with a logit link function) was used as the response variable, and irradiation dose was used as the independent variable. Pairwise comparisons were conducted to examine differences between treatments as appropriate using the package ‘multcomp’ (Hothorn et al., 2017) on R. Death frequency difference between sexes during the mating/inoculation period was analyzed using all tested pairs (300 weevils in each sex) in both species, based on Fisher's exact test. Data from pairs in which a member of the couple died during the mating/inoculation period, or in which the inoculated roots rotted before dissection were removed from the analyses. Furthermore, data from pairs in which sperm was not found in female spermatheca were excluded from the analysis of male fertility. All statistics analyses were conducted using R statistical software version 3.2.3 (R Development Core Team 2015).

Results

Only 248 and 272 treated pairs of *E. postfasciatus* and *C. formicarius*, respectively, were analyzed due to either the death of weevils during the mating/inoculation period (n = 35 and 15, respectively) or to the rotting of sweet-potato roots during the storage period (n = 17 and 13, respectively). Results of irradiation are summarized in Table 1.

Euscepes postfasciatus: In most cases (31 of 35 cases), the dead weevils during the mating/inoculation period (10 days) were females. Death frequency in the mating/inoculation period differed significantly between sexes (male and female, 1.3 and 10%, respectively; Fisher's exact test, $P < 0.001$), but the frequency of male death did not differ significantly between treatments (GLM, Table 1). Male sperm transfer ability was high irrespective of the irradiation treatment (80 to 96%) and there was no significant difference among irradiation treatments (GLMM, Table 1). Above 125 Gy, male fertility was drastically depressed, and tended to decrease with increasing irradiation dose (Table 1). However, males irradiated with

200 Gy still had slight fertility. Three of the 51 males irradiated at this dose produced one progeny. The fertility of male weevils treated with X-rays significantly decreased in relation to that of non-irradiated males.

Cylas formicarius: Death frequency during the mating/inoculation period (seven days) did not significantly differ between sexes (male and female, 2.6 and 2.0%, respectively; Fisher's exact test, $P = 0.788$), and the frequency of male death did not differ significantly between treatments (GLM, Table 1). Male sperm transfer ability was high irrespective of the irradiation treatment (91 to 98%), and there was no significant difference among irradiation treatments (GLMM, Table 1). The fertility of males irradiated with X-ray doses over 100 Gy was completely depressed, as we were not able to detect any progeny by these irradiated males.

Discussion

In recent years, there has been an increasing need for developing an alternative sterilization source to gamma rays (FAO/IAEA 2007; Mastrangelo et al. 2010). The present study indicates that the X-rays generated by the self-contained low-energy X-ray irradiator effectively sterilize mass-reared strains of *E. postfasciatus* and *C. formicarius* at the doses currently used in the eradication programs for these weevils using gamma rays in Japan (*E. postfasciatus*: 150 Gy; *C. formicarius*: 200 Gy, Kuba et al. 2003), and sterilized males have sufficient sperm transfer ability in both weevil species. Dose rate (or dose per unit time) is known to affect the quality of sterilized insects (Barkri et al. 2005; Kumano et al. 2011a,b). For example, Kumano et al. (2011a,b) demonstrated that low dose rate irradiation reduces irradiation damage in *E.*

postfasciatus and *C. formicarius*, and this method has been applied since August 2011 in the eradication program of *E. postfasciatus* using SIT (Kumano 2014). The X-ray dose rates used for each replicate on the present study (*E. postfasciatus*: 7.52 to 7.63 Gy min^{-1}; *C. formicarius*: 6.25 to 6.45 Gy min^{-1}) were lower than the gamma ray dose rates presented in past studies (*E. postfasciatus*: 8.28 to 12.79 Gy min^{-1}; *C. formicarius*: 6.79 to 11.80 Gy min^{-1}, Kumano et al. 2008a,b, 2010a,b, 2011a,b). Thus, the relatively lower dose rate using the X-ray irradiator might reduce fertilization ability without deteriorating sperm transfer ability in these weevils, as observed for gamma rays.

The physical properties of X-rays are different from that of gamma rays; X-rays have wider wavelength range and lower permeability than gamma rays from ^{60}Co (e.g., Bakri et al. 2005). The X-rays generated by the X-ray irradiator are as biologically effective as gamma rays for SIT in dipteran species (Mastrangelo et al. 2010), and the small-scale study presented here demonstrated that the X-rays generated using the low-energy X-ray irradiator could be used to sterilize a small number of weevils in Petri dishes. However, there is concern that the relatively thicker exoskeleton of adult weevils compared to the pupa of dipteran species might adversely affect dose uniformity when a large number of individuals are placed in the irradiation canister to induce sterilization by using low penetration X-rays. Achieving dose uniformity in the canister used for a large scale irradiation is important for the practical application of low-energy X-ray irradiation in SIT considering the various physical and biological factors. Additional studies of SIT for the control of weevils are therefore needed to confirm dose uniformity in the large irradiation canister.

Eradication programs currently in progress for *C. formicarius* use 200 Gy by gamma rays as the irradiation dose (Kuba et al. 2003). However, previous studies on sterilization using gamma rays reported that *C. formicarius* males irradiated at 100 Gy at the adult stage almost completely lost their fertilization ability in both small- and large-scale tests (small-scale test: Kumano et al. 2010a; large-scale test: Sharp 1995). Thus, the dose used to obtain full sterilization in the eradication programs using gamma rays for *C. formicarius* in Japan (200 Gy) might be too high even taking account of the risk of male's residual fertility. Bakri et al. (2005) suggested that full sterility is not a favorable condition for the program, and thus process optimization is necessary to balance sterility level and competitiveness. For example, partial sterilization is useful in the early stages of the eradication program (Knipling, 1955; Suzuki & Miyai, 2000). Therefore, further studies should evaluate the relationship between male sperm transfer ability and the degree of partial sterilization using the X-ray irradiator for *C. formicarius* in large-scale tests.

Previous studies demonstrated that the sperm transfer ability of individuals sterilized by gamma rays from ^{60}Co decreases drastically over time in *E. postfasciatus* and *C. formicarius* (Kumano et al. 2008a,b). Although these negative effects may also occur in weevils sterilized by X-ray irradiation, temporal changes in sperm transfer ability after sterilization and in male mating competitiveness with non-irradiated (wild) males were not investigated in the present study. Thus, future studies should aim to clarify these changes after irradiation. Kumano et al. (2010a,b; 2011a,b) demonstrated that fractionated-dose irradiation and partial sterility improve the sperm transfer ability of irradiated weevil males. These techniques would also be effective in the practical application of the low-energy X-ray irradiator in future SIT programs.

Acknowledgements

We thank Mr. Yutaka Nakamoto, Mr. Yoshitaka Sokei (Okinawa Prefectural Plant Protection Center), and Dr. Goro Matsuzaki (TBRCUR) for technical advice on irradiation. We also thank the staff at OPARC for their assistance with insect rearing.

References

Bakri A, Mehta K, Lance D (2005) Sterilizing insects with ionizing radiation. In: Dyck VA, Hendrichs J, Robinson AS (eds) Sterile insect technique: principles and practice in area-wide integrated pest management, Springer, Dordrecht, pp 233–268

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127-135

Calkins CO, Parker AG (2005) Sterile insect quality. In: Dyck VA, Hendrichs J, Robinson AS (eds) Sterile insect technique: principles and practice in area-wide integrated pest management, Springer, Dordrecht, pp 269–296

Chalfant KL, Jansson RK, Seal DR, Schalk JM (1990) Ecology and management of sweet potato insects. Annu Rev Entomol 35:157–180

Downey D, Chun S, Follett PA (2015) Radiobiology of small hive beetle (Coleoptera: Nitidulidae) and prospects for management using sterile insect releases. J Econ Entomol 108:868-872

Dyck, VA, Hendrichs J, Robinson AS. (2005). Sterile insect technique: principles and practice in area-wide integrated pest management (Eds). Springer, Vienna

FAO/IAEA (2007) Insect pest control newsletter 68. International Atomic Energy Agency, Vienna. Pp.1-3. http://www-pub.iaea.org/MTCD/Publications/PDF/Newsletters/IPC-NL-68.pdf

FAO/IAEA (2008) Insect pest control newsletter 70. International Atomic Energy Agency, Vienna. Pp.1-4. http://www-pub.iaea.org/MTCD/Publications/PDF/Newsletters/IPC-NL-70.pdf

FAO/IAEA (2017) Insect and pest control newsletter 88. International Atomic Energy Agency, Vienna. Pp.1-3. http://www-pub.iaea.org/MTCD/Publications/PDF/Newsletters/IPC-88.pdf

Fisher K (1997) Irradiation effects in air and in nitrogen on Mediterranean fruit fly (Diptera: Tephritidae) pupae in Western Australia. J Econ Entomol 90:1609–1614

Follett PA (2006) Irradiation as a methyl bromide alternative for postharvest control of *Omphisa anastomosalis* (Lepidoptera: Pyralidae) and *Euscepes postfasciatus* and *Cylas formicarius elegantulus* (Coleoptera: Curculionidae) in Sweet Potatoes. J Econ Entomol 99:32–37

Hallman GJ (2003) Ionizing irradiation quarantine treatment against plum curculio (Coleoptera: Curculionidae). J Econ Entomol 96:1399–1404

Hayashikawa, S. (2005) Occurrence and protection of the sweetpotato weevil *Cylas formicarius* in Amami Islands. Jpn Agric Tech 49:35–39.

Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S, Hothorn MT (2017). Package ‘multcomp’. http://ftp5.gwdg.de/pub/misc/cran/web/packages/multcomp/multcomp.pdf

Jansson RK, Raman KV (1991) Sweet potato pest management: a global overview. In: Jansson RK, Raman KV (eds) Sweet potato pest management: a global perspective. Westview Press, Boulder, pp 1–12

Knipling EF (1955) Possibilities of insect control or eradication through the use of sexually sterile males. J Econ Entomol 48:459–462

Kohama T, Sugiyama M (2000) External characteristics for sexing the West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae). Entomol Sci 3:43–45

Kuba H, Kohama T, Haraguchi D (2003) Eradication projects of exotic sweet potato weevils using SIT in Okinawa. In: Oka M, Matsui M, Shiomi T, Ogawa Y, Tsuchiya K (eds) Proceedings of the NIAES-FFTC joint international seminar on biological invasions: environmental impacts and the development of a database for the Asian-Pacific region. National Institute for Agro-Environmental Sciences and Food and Fertilizer Technology Center for the Asian and Pacific Region, Tsukuba, 273–287

Kumano N. (2014) Recent advances in the study on sterile technique for the West Indian Sweet Potato Weevil, *Euscepes postfasciatus*. Jpn J Appl Entomol Zool 58:217-236

Kumano N, Haraguchi D, Kohama T (2008a) Effect of irradiation on mating performance and mating ability in the West Indian sweet potato weevil, *Euscepes postfasciatus*. Entomol Exp Appl 127:229–236

Kumano N, Haraguchi D, Kohama T (2008b) Effect of irradiation on mating ability in the male sweetpotato weevil (Coleoptera: Curculionidae). J Econ Entomol 101:1198–1203

Kumano N, Kuriwada T, Shiromoto K, Haraguchi D, Kohama T (2010a) Assessment of effect of partial sterility on mating performance in sweetpotato weevil (Coleoptera: Curculionidae). J Econ Entomol 103:2034–2041

Kumano N, Kuriwada T, Shiromoto K, Haraguchi D, Kohama T (2010b) Evaluation of partial sterility in mating performance and reproduction of the West Indian sweet potato weevil, *Euscepes postfasciatus*. Entomol Exp Appl 136:45–52

Kumano N, Kuriwada T, Shiromoto K, Haraguchi D, Kohama T (2011a) Fractionated irradiation improves the mating performance of the West Indian sweet potato weevil *Euscepes postfasciatus*. Agric Forest Entomol 13:349–356

Kumano N, Kuriwada T, Shiromoto K, Haraguchi D, Kohama T (2011b) Prolongation of the effective copulation period by fractionated dose irradiation in the sweet potato weevil, *Cylas formicarius*. Entomol Exp Appl 141:129-137

Lance DR, McInnis DO (2005) Biological basis of the sterile insect technique. In: Dyck VA, Hendrichs J, Robinson AS (eds) Sterile insect technique: principles and practice in area-wide integrated pest management, Springer, Dordrecht, pp 69–94

Mastrangelo T, Parker AG, Jessup A, Pereira R, Orozco-d Vila D, Islam A, Dammalage T, Walder, J (2010) A new generation of X ray irradiators for insect sterilization. J Econ Entomol 103:85–94

Mehta K, Parker A (2011) Characterization and dosimetry of a practical X-ray alternative to self-shielded gamma irradiators. Rad Phys Chem 80:107–113

Miyaji K, Nishihara S, Hara Y, Tokunaga T, Hatono T, KamikadoT, Ito S, Iwamoto J, Aramaki Y, Kinjyo K, Sokei Y(2000) Eradication of weevils by sterile-re- lease method (6) Methods of mass rearing, sterilization, marking, and release of *Cylas formicarius.* Plant Prot 54:472–475 (In Japanese)

Okinawa Prefectural Government, Department of Agriculture, Forestry and Fisheries **(**2013**)** *Annual report of eradication program for the destructive pest in Okinawa in 2011*. Okinawa Pref Gov, Dep Agric, Forest and Fish, Okinawa, Japan. 350 pp. (In Japanese)

Raman KV, Alleyne EH (1991) Biology and management of the West Indian sweet potato weevil, *Euscepes postfasciatus*. In Jansson RK, Raman KV (Eds.), *Sweet potato pest management*. Boulder, USA: Westview Press, pp. 263–281

R Development Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Sakurai H (2000) Eradication of weevil by sterile-insect-release method (4) physiology of reproduction in sterilized insects. Plant Prot 54:466–468 (In Japanese)

Sakurai H, Watanabe H, Sugimoto T, Sakuratani Y, Setoguchi O, Kawazoe K (1994) Effect of gamma-radiation on the gametogenesis in the sweetpotato weevil, *Cylas formicarius.* Res Bull Facul Agric Gifu Univ 59:11–20 (in Japanese with English Summary)

Sakurai H, Murakami Y, Kohama T, Teruya T (2000a) Sterilizing mechanism of gamma-radiation in the female of West Indian sweet potato weevil, *Euscepes fasciatus*. Res Bull Facul Agric Gifu Univ 65:13–20 (in Japanese)

Sakurai, H, Murakami Y, Uchimura H, Kohama T, Teruya T (2000b) Sterilizing mechanism of gamma-radiation in the male of West Indian sweet potato weevil, *Eusceps postfasciatus*. Res Bull Facul Agric Gifu Univ 65: 5-12 (in Japanese)

Sharp J (1995) Mortality of sweet potato weevil (Coleoptera: Apionidae) Stages Exposed to Gamma Irradiation. J Econ Entomol 88:688–692

Sherman M, Tamashiro M (1954) The sweetpotato weevils in Hawaii: their biology and control. Hawaii Agric Exp Station Res Bull 23:1–36

Suzuki, Y, Miyai S (2000) Eradication of weevils by sterile-insect release method (5): Making use of partially sterilized insects. Plant Prot 54: 469-470 (In Japanese)

United States Food and Drug Administration (2004) Irradiation in the production, processing and handling of food: final rule. Fed Reg 69: 76844–76847

Yamada, H, Parker, AG, Oliva, CF, Balestrino, F, Gilles, JRL (2014). X-ray-induced sterility in *Aedes albopictus* (Diptera: Culicidae) and male longevity following irradiation. J Med Entomol 51: 811-816.

Yasuda K (1993) Seasonal prevalence of the sweetpotato weevil *Cylas formicarius* and the West Indian sweetpotato weevil *Euscepes postfasciatus* in Okinawa, Japan. Proc Assoc Plant Prot Kyushu 39:88–90 (in Japanese)

Yasuda K, Kohama T (1990) Distribution of the sweetpotato weevil, *Cylas formicarius* (Fabricius) and the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmire) in Okinawa prefecture. Proc Assoc Plant Prot Kyushu 36:123–125 (in Japanese)

Table 1 Effects of X-ray irradiation generated by low-energy self-contained X-ray irradiator on sperm transfer ability and fertility in the West-Indian sweetpotato weevil *Euscepes postfasciatus* and in the sweetpotato weevil *Cylas formicarius.*

Species	Irradiation dose	Tested pair	Eriminated pair from data analysis		Analyzed pair	No. of sperm transfer success (success rate [%])[2]	Descriptive statistic for progeny Mean±SE, (Max., min.)			
	(Gy)	n	Death of weevil, n (male[1], female)	Rotted roots, n	n		Adult	Pupae	Lavae	Total[3]
Euscepes postfasciatus										
	0	60	5 (2, 3)	5	50	42 (80)	9.5±1.4, (31, 0)	0.0±0.0, (1, 0)	0.0±0.0, (0, 0)	9.8±0.4, (31, 0) a
	125	60	12 (1, 11)	4	44	39 (89)	0.3±0.1, (3, 0)	0.0±0.0, (1, 0)	0.0±0.0, (0, 0)	0.3±0.1, (3, 0) b
	150	60	4 (0, 4)	2	54	52 (96)	0.3±0.1, (2, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.2±0.7, (2, 0) bc
	175	60	10 (0, 10)	3	47	44 (94)	0.0±0.0, (1, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.0±0.0, (1, 0) c
	200	60	4 (1, 3)	3	53	51 (96)	0.0±0.0, (1, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.1±0.0, (1, 0) c
	Subtotal	300	35 (4,31)	17	248	228 (92)	2.0±0.4, (31, 0)	0.0±0.0, (1, 0)	0.0±0.0, (1, 0)	2.0±0.4, (31, 0)
Cylas formicarius										
	0	60	7 (2, 5)	1	52	50 (96)	8.0±0.7, (22, 0)	0.0±0.0, (1, 0)	0.7±0.1, (4, 0)	7.3±0.7, (22, 0)
	100	60	1 (1,0)	4	55	50 (91)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)
	150	60	1 (1,0)	3	56	52 (93)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)
	200	60	2 (2, 0)	3	55	53 (96)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)
	250	60	4 (4, 0)	2	54	53 (98)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)	0.0±0.0, (0, 0)
	Subtotal	300	15 (10, 5)	13	272	258 (95)	1.5±0.2, (22, 0)	0.0±0.0, (1, 0)	0.1±0.2, (4, 0)	1.4±0.2, (22, 0)

[1] There was no significant difference among treatments in the frequency of male death of both species.

[2] The frequency of sperm transfer ability in both species was not significantly different among treatments.

[3] The total progeny numbers were not significantly different ($P > 0.05$) among the values accompanied by the same lower-case letters.

Legends to Figures

Fig. 1 The low-energy self-contained X-ray irradiator (MBR-1505R-2, Hitachi, Tokyo, Japan). Whole irradiator (*a*) and its irradiation chamber (*b*).

Fig. 2 Position of the Petri dishes in the irradiation chamber during irradiation. View from the side (*a*) and from above (*b*).

a)

b)

Fig. 1

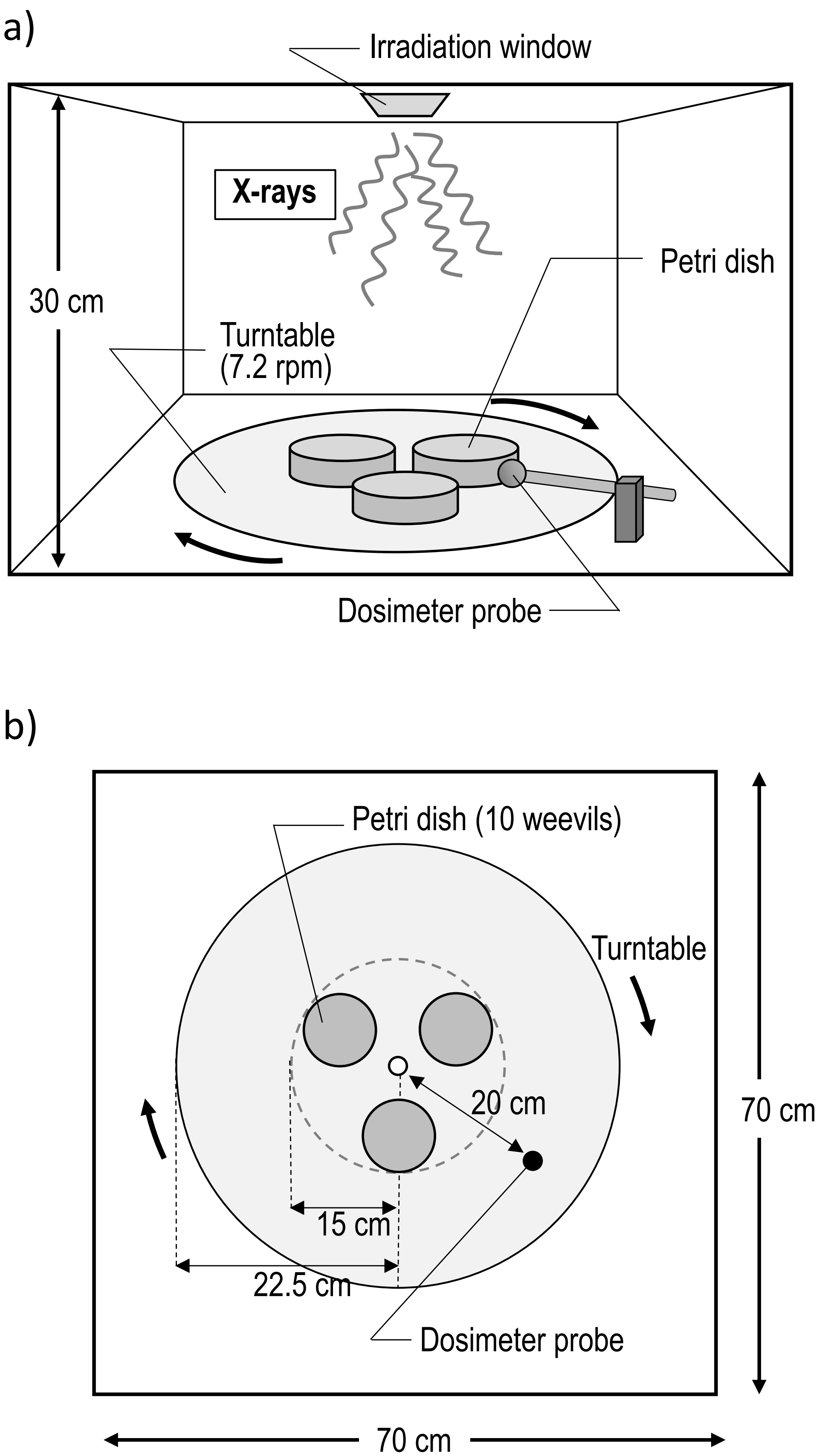
a)
Irradiation window
X-rays
Petri dish
30 cm
Turntable
(7.2 rpm)
Dosimeter probe
b)
Petri dish (10 weevils)
Turntable
20 cm
70 cm
15 cm
22.5 cm
Dosimeter probe
70 cm

Fig. 2