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Developmental inhibition of gamma irradiation on the peach fruit moth *Carposina sasakii* (Lepidoptera: Carposinidae)

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HIGHLIGHTS

- Effects of gamma irradiation were tested on *Carposina sasakii.*
- LD99 values for the inhibition of adult emergence was 191.0 Gy.
- Irradiation of infested apples at 150 Gy completely inhibited the emergence of next generation.

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ABSTRACT

Ionizing irradiation is a useful technique for disinfestation under plant quarantine as well as post-harvest management. Effects of gamma irradiation treatment were tested on different developmental events of *Carposina sasakii*, which is a serious pest of various orchard crops. Apple fruits infested by *C. sasakii* were irradiated by gamma rays ranging from 0 to 300 Gy. Inhibition rates were determined on behavioral events related to development, including larval exit from apples, cocoon formation, adult eclosion, and oviposition. Failure rates of all these developmental events increased with increasing doses of irradiation. Rates of larval exit from apples and cocoon formation decreased to 13.2% and 1.7%, respectively, at 300 Gy. However, the adult eclosion rate decreased to 5.4% at 100 Gy and was completely inhibited at doses greater than 150 Gy. LD99 values for the inhibition of cocoon formation and adult emergence was estimated into 313.4 and 191.0 Gy. Furthermore, adults developed from irradiated larvae completely failed to lay eggs. Thus, irradiation of infested apples at doses of 200 Gy and higher completely inhibited the next generation of *C. sasakii*. Our results suggest that gamma irradiation treatment would be a promising technique for the control of *C. sasakii*.

1. Introduction

Peach fruit moth, *Carposina sasakii* Matsumura, is a serious pest of various kinds of ornamental fruits, including apple, pear, and peach. Due to its restricted distribution in eastern Asia, including Japan, China, Korea, and the Far-eastern region of Russia, *C. sasakii* is considered as an important plant quarantine pest (Narita, 1986). Pesticide control of *C. sasakii* is difficult due to its infestation behavior into the inside of fruit (Kim and Lee, 2002). Similar to the codling moth *Cydia pomonella*, the first instar larvae bore into fruit and grow inside until the last instar larvae. Thus, chemical control should be conducted before infestation of hatched larvae into fruit. Its adult emergence appears two times a year but widely spreads within several months from late May to the mid-September, making control using pesticides very difficult (Kim et al., 2001). Recently, sex pheromones in the form of pheromone traps or mating disruptors were shown to be useful to control this pest (Han et al., 2000; Nasu et al., 2010). However, due to its status as a quarantine pest, infestation, even in a small scale, disrupts exports to foreign countries.

Ionizing radiation has been suggested as a useful alternative to the fumigation of methyl bromide, which has been used as a major post-harvest quarantine treatment but with harmful effects on human health and the environment as a significant ozone-depleting substance (Ross, 1999; Hallman, 2011). Otherwise, ionizing radiation has some advantages, such as non-development of insect resistance, absence of residue in treated foods, and insignificant loss of nutrients in commodities (Lapidot et al., 1991; Marcotte, 1993; Hallman and Martinez, 2001; Kwon et al., 2004). Whereas gamma irradiation at a dose of at least 1 kGy dose can completely disinfest most pest insects, it causes damage to most fresh agricultural commodities (Hallman, 2011). Therefore, it is

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necessary to reduce irradiation doses in commercial applications. Although high doses of irradiation could immediately kill insects, normal growth, development, or reproduction of the organism might be prevented by lower doses of irradiation (Hallman, 2012). Tolerance to gamma radiation is known to vary among species as well as according to developmental stage even within the same species (Cogburn et al., 1973; Burditt et al., 1989; Ozyardimci et al., 2006; Hallman et al., 2010). Therefore, it is important to determine the irradiation tolerance of each developmental stage of the target insect as well as the effective irradiation dose for the useful control of pests (IAEA/IDIDAS, 2006).

The purpose of this study was to determine the inhibitory doses of gamma irradiation treatment on several important events of behavior and development in *C. sasakii*. Our results provide information for the practical use of gamma irradiation for the control of *C. sasakii*.

2. Materials and methods

2.1. Insects

Apple fruits were collected in early July from 2010 to 2012 at pesticide-free orchards in Youngcheon County, Korea. Apples were 5–6 cm in diameter, and most apples were irregularly shaped, which is one symptom of infestation by *C. sasakii*. Collected apples were washed in a solution of 0.1% methylparaben and air-dried. Apples were kept at a weight of 14 kg in a plastic tray ($42 \times 32 \times 17$ cm^3) covered with a fine meshed net, which was maintained in a rearing room under conditions of 25 ± 2 °C, 70 ± 5% relative humidity, and a 16 h light:8 h dark (16L:8D) photoperiodic cycle. Matured larvae that escaped from the apples were transferred into a plastic box ($19 \times 12 \times 11$ cm^3) containing sterilized sand soils that retained moisture from water spraying every 3 days. Cocoons were collected and transferred into a new plastic box to allow adult eclosion. Adults were collected within 1 day after eclosion, after which five pairs of adults were transferred into a new plastic cage ($28 \times 21 \times 15$ cm^3) containing 3–5 fresh apple fruits to allow mating and oviposition. Eggs laid in apples were maintained under the same conditions as previously described.

2.2. Treatment by gamma irradiation

For the irradiation experiment, apples were collected from the orchards in early July. Some apples ($n=100$) were dissected and the developmental stage of infested *C. sasakii* was identified. Gamma irradiation was conducted at the Korea Atomic Energy Research Institute (KAERI), Jeongeup, Korea using a Co^{60} source (AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) at a dose rate of 1 kGy/h. Apples (14 kg/box) infested by *C. sasakii* at apple orchards were treated with doses of 0, 100, 150, 200, 250, and 300 Gy. Target doses were monitored by a free radical and ceric/cerous dosimeter (± 5.6%). After irradiation, apples were maintained under the previously described conditions. Effects of gamma radiation on developmental events, including larval exit from apples, cocoon formation, and adult eclosion, were determined. We recorded the number of cocoons rather than pupation since it was not possible to directly observe the pupae inside the cocoon. Further oviposition rates were determined from emerged adults. The number of larvae that exited from non-irradiated apples were considered as the control and compared with numbers of individuals in irradiated apples at each developmental event.

2.3. Data analysis

A randomized design was performed with six treatments (Gy) and three replicates. Linear regression analyses were used for evaluating the relationship between various irradiation doses, and cocoon formation and adult eclosion were calculated following Probit analysis (Finney, 1971). Data of Probit analysis was used to determine the LD90 and LD99 values using the Statplus program (2009). The results were registered as inhibitory rates (%). The experiment was repeated three times using the larvae collected over three years.

3. Results and discussion

Ionizing radiation is a useful alternative against methyl bromide fumigation for quarantine of fruits infested by *C. sasakii*. Our results showed that gamma irradiation of apples infested by larvae of *C. sasakii* significantly inhibited rates of further behaviors and development, such as larval exit, cocoon formation, and adult eclosion.

The target stage for irradiation of apples was the larval stage of *C. sasakii*. We identified more than 80% of infested *C. sasakii* was the fifth instar larval stage from collected apples in the orchards in early July. The first exit of larvae from the apples was appeared at 7 days after treatment. Total number of larvae that exited from the non-irradiated apples in 3-years experiment was 3045 individuals. We used this number as the control in order to compare developmental inhibition of irradiated groups. In comparison with the control, the larval exit rate from apples gradually decreased when irradiation doses are increased in the range from 0 to 300 Gy (Table 1). The exit rate was 87.0% at 100 Gy of irradiation but was reduced to 13.2% of the control at 300 Gy. Effect of gamma irradiation was determined according to the time and duration of larval exit from apples. Most larvae exited between 7 and 22 days after irradiation and peaked at 12–17 days (Fig. 1). However, the time of larval exit was slightly delayed by irradiation and peaked at 17 days. This suggests that the exit behavior could be significantly damaged by irradiation.

Further, cocoon formation was 90.7% in the control but decreased to 56.8% and 1.7% by irradiation at 100 Gy and 300 Gy,

Table 1
Effects of gamma irradiation on the inhibition of behavioral and developmental events of *Carposina sasakii*.

Developmental events	Inhibitory rates (%) by treatments of various irradiation doses (Gy)						LD90 (95% CI)	LD99 (95% CI)
	0	100	150	200	250	300		
Larvae that exit the fruits	100*	87.0 ± 6.1	69.0 ± 13.2	46.1 ± 16.5	26.6 ± 12.6	13.2 ± 8.1		–
Cocoon formation	90.7 ± 6.2	56.8 ± 7.5	41.6 ± 20.0	24.2 ± 16.5	11.5 ± 10.3	1.7 ± 1.7	262.8 (242.5; 283.1)	313.4 (293.1; 333.7)
Adult eclosion	39.7 ± 5.2	5.4 ± 1.3	0	0	0	0	133.7 (127.4; 139.9)	191.0 (184.7; 197.2)

* As a control, larvae that exit from non-irradiated apples (14 kg) were counted as 1015 individuals in each year (total 3045 in 3 years) and this number considered as 100% of larvae that exit the fruits in each group. Total numbers escaped from irradiated apples were estimated from the numbers of collected larvae in the control. The percentages of further cocoon formation and adult eclosion were calculated based on the numbers of the escaped individuals of the control.

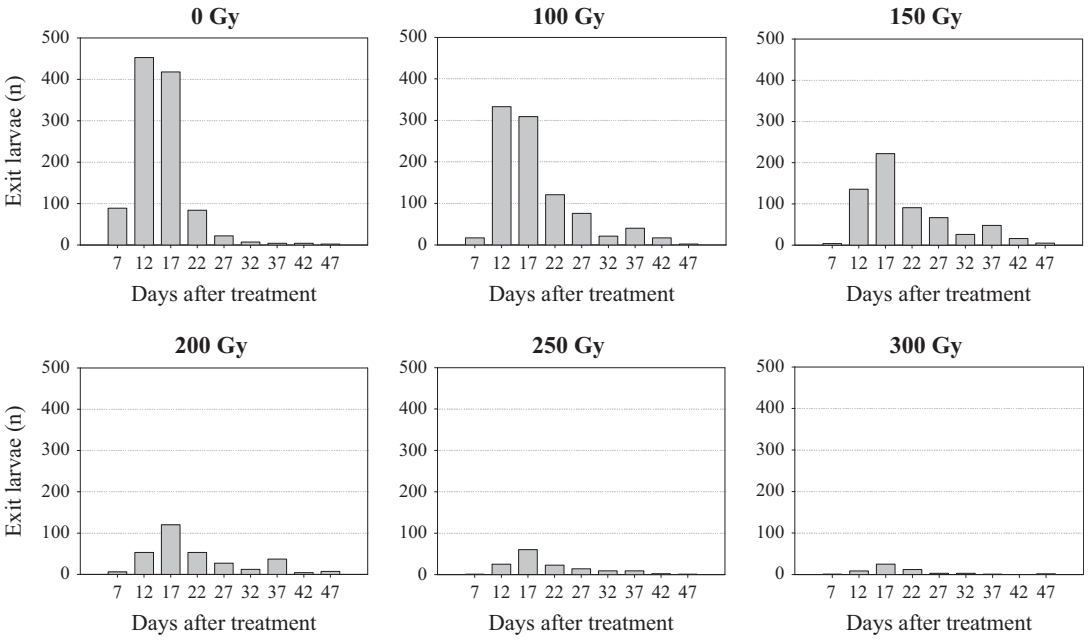

Fig. 1. Number of larvae exit from apple fruits after treatment with different doses of gamma irradiation. Apples infested by *Carposina sasakii* were irradiated at different doses of 0, 100, 150, 200, 250, and 300 Gy and then incubated for 50 days under room temperature conditions until larval exit behaviors were completed.

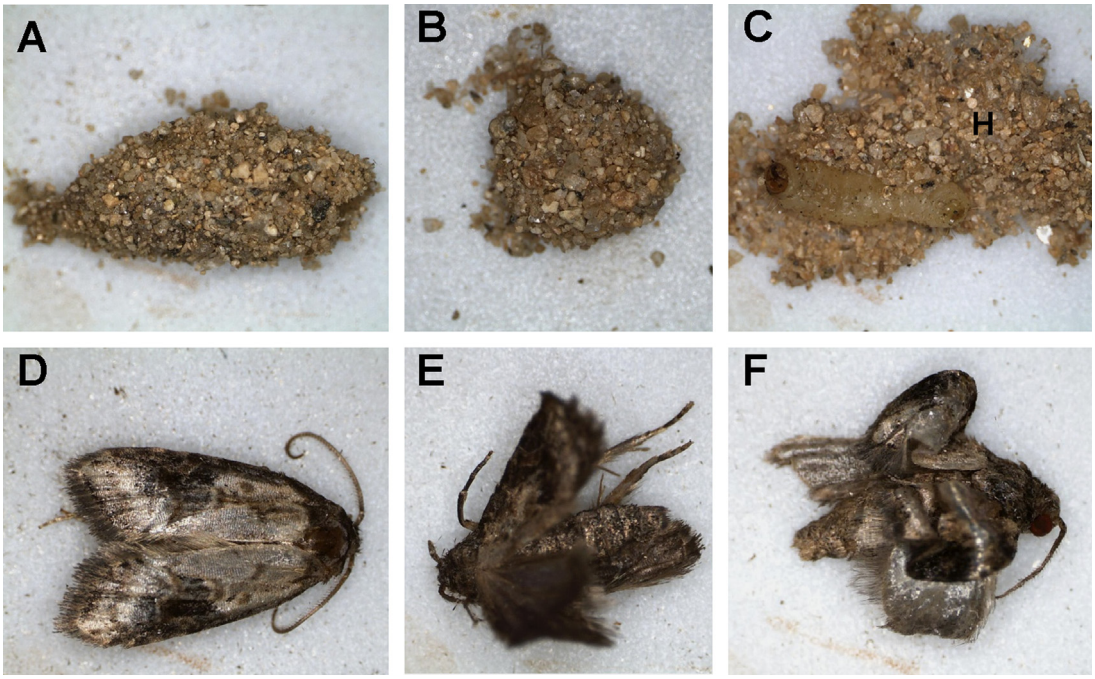

Fig. 2. Morphological abnormality of *Carposina sasakii*. Cocoon formation was observed in individuals that exit from non-irradiated (A) and irradiated apples at 250 Gy (B) and 300 Gy (C). Eclosed adults were observed in individuals that exit from non-irradiated (D) and irradiated apples at 100 Gy (E and F).

respectively (Table 1). Cocoon formation occurred under the soil in an oval shape upon non-irradiation (Fig. 2A), whereas its shape was abnormal or not completely formed at higher doses of irradiation (Fig. 2B and C). Pupation of *C. pomonella* can be prevented when fifth instar larvae are irradiated at 230 Gy (Proverbs and Newton, 1962). Likewise, pupation of *Plodia interpunctella* does not occur when fifth instar larvae are irradiated at 250 Gy and higher doses (Aye et al., 2008). In *Spodoptera litura*, irradiation at 100 Gy or higher is enough to inhibit pupation (Dohino et al., 1996). Our results showed that the LD99 value for the inhibition of cocoon formation was 313.4 Gy (Table 1). This value is slightly higher than those of other lepidopteran insects. However, this may be due to the differences on irradiation methods because we irradiated infested apples by *C. sasakii* larvae rather than direct treatment into individuals.

Adult eclosion from control larvae was 39.7% but only 5.4% at 100 Gy and 0% at 150 Gy and higher. The bodies of a few emerged adults were highly deformed, particularly their wings and legs (Fig. 2E and F). Moreover, these abnormal adults cannot be able to undergo successful copulation and oviposition. Thus, adult emergence was totally inhibited at doses of 150 Gy and higher. Our results showed that the LD99 value for the inhibition of adult

emergence was 191.0 Gy (Table 1). In a similar experiment on *C. pomonella*, adult eclosion was shown to be less than 2% at 150 Gy and completely prevented at 200 Gy (Mansour, 2003). Adult emergence of oriental fruit moth *Grapholita molesta* can be inhibited by irradiation at 200 Gy under ambient atmosphere and at 232 Gy under hypoxic atmosphere (Hallman, 2004). The USDA allows irradiation at 400 Gy for all non-reviewed insects except pupae and adults of Lepidoptera (APHIS, 2006). International Standards for Phytosanitary Measures were applied for the irradiation of fruits and vegetables at a minimum absorbed dose of 200 Gy in order to prevent the adult emergence of this species at the stated efficacy (ISPM, 2009).

Prevention of adult emergence is the criterion for effectiveness in terms of control of fruit pathogens. Recently, Hallman et al. (2013) summarized that 250 Gy as a preventive dose for adult emergence is supported by many studies comprising 34 species in 11 families of Lepidoptera. In our case, irradiation at 200 Gy was enough to prevent adult emergence of *C. sasakii*. Our results suggest that apples infested by *C. sasakii* can be disinfested by irradiation at a similar level as other fruit-boring insects.

Guidelines for the practical use of irradiation have been prepared as a phytosanitary treatment for regulated pests (ISPM, 2003). Further research is needed to achieve international acceptance of 200 Gy as a quarantine treatment for *C. sasakii*. For example, the irradiation efficacy can be changed by some factors such as oxygen concentration, host, temperature, dose rate and pest phenotype (Hallman et al., 2010). We have to demonstrate the effects of physiological and environmental factors on the irradiation efficacy of *C. sasakii* in quarantine disinfestations.

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