Effects of irradiation of each of the five peach fruit moth (Lepidoptera: Carposinidae) instars on 5th instar weight, larval mortality and cumulative developmental time: A preliminary investigation

Baishu Li[1], Meixu Gao[2], Bo Liu[1], Tianxu Li[1], Yuejin Wang[1,], and Guoping Zhan[1,*]*

Abstract

The peach fruit moth, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), is a serious pest of many pome and stone fruits and presents a quarantine problem in some export markets. To investigate the effects of irradiation on larval weight, developmental time, and mortality, 3-, 6-, 9-, 12-, and 15 d-old larvae—which were 1st, 2nd, 3rd, 4th and 5th instars, respectively—were reared within 'Red Fuji' apple (*Malus domestica* Borkh.; Rosales: Rosaceae) fruits, and gamma-irradiated at doses from 20 to 140 Gy in increments of 20 Gy. Irradiation had significant effects on larval weight, cumulative larval developmental time, and mortality. More specific findings were as follows. *Body Weight.* The mean body weight of late 5th instars that developed from the 4 preceding instars decreased significantly in a dose-dependent manner when larvae in any one of these preceding instars had been irradiated with gamma rays. Mean body weight reduction of late fifth instars was greatest when 3rd instars were irradiated. In contrast, when 5th instars were irradiated their mean body weight increased significantly. *Cumulative Larval Development Time*. The mean cumulative larval developmental times increased significantly in a dose-dependent manner in comparison to the controls when any one of the 5 instars was irradiated. *Larval Mortality.* Significant larval mortality occurred in the first 4 instars when any one of them had been irradiated, but none of the irradiated 5th instars died in the apple fruits, indicating that the 5th instar is the most radiotolerant larval stage. *Additional Research*. Further research is suggested to elucidate the mechanisms whereby the weights of 5th instars that developed from irradiated 3rd instars are substantially diminished. Also research is suggested to elucidate the mechanisms whereby the weights of irradiated 5th instars increase substantially after they have been irradiated.

Key Words: peach fruit moth; *Carposina sasakii*; irradiation; radiation effect; weight; developmental time; mortality

Resumen

La polilla del fruto del melocotón, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), es una plaga seria de muchas frutas de pepita y de pomelos y representa un problema de cuarentena en algunos mercados de exportación. Para investigar los efectos de la irradiación sobre el peso de las larvas, el tiempo de desarrollo, y la mortalidad, larvas de 3, 6, 9, 12 y 15 horas de edad, las cuales fueron de 1º, 2º, 3º, 4º y 5º estadio, respectivamente—fueron criadas en la fruta de manzana 'Fuji' (*Malus domestica* Borkh.: Rosales: Rosaceae) y gamma-irradiadas a los dosis de 20-140 Gy en incrementos de 20 Gy. La irradiación tuvo efectos significativos sobre el peso de las larvas, el tiempo total de desarrollo de las larvas y la mortalidad. Los hallazgos más específicos son los siguientes. *Peso Corporal.* El promedio del peso corporal de los 5º estadios mayores que se desarrollaron a partir de los 4 estadios anteriores disminuyó significativamente en una manera dependiente de la dosis cuando las larvas en cualquiera de estos estadios previos habían sidos irradiadas con rayos gamma. La reducción del peso corporal de los quinto estadios tardios fue mayor cuando se irradiaron los tercer estadios de larvas. En contraste, cuando se irradiaron los 5º estadios, su peso medio corporal aumentó significativamente. *Tiempo total de desarrollo de larvas*. El promedio del tiempo total de desarrollo de larvas aumentaron significativamente en una manera dependiente de la dosis en comparación con los controles cuando cualquiera de los 5 estadios fueron irradiados. *Mortalidad de larvas*. Mortalidad significativa de larvas sucedio en los primeros 4 estadios, cuando uno de ellos había sido irradiado, pero ninguna de las larvas del 5º estadio irradiadas murió en las manzanas, lo que indica que el 5º estadio es el estadio larval más radio-tolerante. *Necesita investigación adicional*. Se necesitan más investigaciones para dilucidar los mecanismos por los que el peso de larvas irradiadas del 5º estadio que se desarrollaron a partir de larvas del 3º estadio irradiado disminuyeron considerablemente. También se necesita investigación para dilucidar el mecanismo por el cual el peso de las larvas del 5º estadio se aumentó sustancialmente después de que han sido irradiados.

Palabras Clave: polilla del melocotón; *Carposina sasakii*; irradiación; efecto de la radiación; peso; tiempo de desarrollo; mortalidad

The peach fruit moth, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), is one of the most destructive borers of pome and stone fruits, including apple (*Malus* spp.; Rosales: Rosaceae), pear, peach, plum, apricot (all *Prunus* spp.; Rosales: Rosaceae), Chinese quince (*Chaenomeles sinensis* (Thouin) Koehne; Rosales: Rosaceae: Amygdaloideae), jujube also known Chinese date (*Zizyphus jujuba* Mill.; Rosales: Rhamnaceae), and hawthorn (*Crataegus* spp.; Rosales: Rosaceae: Amygdaloideae) in China (Hunag & Wu 1975; EPPO 1997; Li et al.

[1]Chinese Academy of Inspection and Quarantine, Beijing, 100029, P. R. China
[2]Institute of Agro-products Processing Science & Technology, Chinese Academy of Agricultural Science, Beijing, 100193, P. R. China
*Corresponding authors: E-mail: wangyj@caiq.gov.cn; zhgp136@126.com

2012a). It is widely distributed in pome fruit production areas in China, Japan, both Koreas, and the Far Eastern Federal District of Russia (Hua 1992; EPPO 1997). Peach fruit moth is considered as an important plant quarantine pest, and its spread may be prevented by applying a phytosanitary treatment to the fruits before export.

Currently, methyl bromide fumigation is the only effective phytosanitary treatment of host fruits infested by *C. sasakii*, but fumigation leaves residues (Bell 2000; Li et al. 2012b). Irradiation is a promising phytosanitary treatment that is increasing in use worldwide. Advantages over other treatments include tolerance by the vast majority of fresh commodities, ability to treat the commodity in its final packaging in quantities as large as pallet loads, and lack of residues (Hallman 2011). Use of radiation to prevent adult emergence and the minimum dose needed to provide quarantine security against *C. sasakii* eggs and against the stages within the fruit (instars 1–5) have been reported (Zhan et al. 2013, 2014a, 2014b; Ryu et al. 2015). Also the effect of irradiation in preventing the hatch of F_1 eggs laid by adults that emerged from irradiated pupae was investigated by Zhang & Wang (1983) and Zhan et al. (2014c). The aim of this research was to investigate the effects of gamma-irradiation of each of the 5 instars on the weights of 5th instars and the cumulative developmental time of instars 1–5, because relevant information is absent from the literature.

Materials and Methods

INSECT REARING

Carposina sasakii insects were allotted from the original culture that infested apples growing in Hebei Province, China and were reared as Liu et al. (2010) described. All instars of *C. sasakii* were reared in apples at 24–26 °C, 50-70% RH and 15:9 h L:D in the laboratory. Late 5th instars that emerged from infested apples were weighed with a precision of ± 0.1 mg and transferred to moist pine sawdust for pupation and eclosion. Paired adults (10♀:12♂) were fed a 5% honey water solution through a wick. The females laid eggs on rough filter papers. Papers with adhering 5d-old eggs were wetted and stuck to the calyx end of "Red Fuji" apples and placed on a steel tray and sealed with a thin plastic sheet for 3 days. The neonates that hatched from the 5d-old eggs penetrated into the apples where the subsequent instars developed until the late fifth instars emerged from the infested apples (Zhan et al. 2014b).

First, 2nd, 3rd, 4th and 5th instars were obtained for these experiments by infesting 5d-old eggs on "Red Fuji" apples (15~20 eggs per apple) for 3, 6, 9, 12, and 15 d before irradiation, respectively. Equal numbers of eggs were used to infest each of the apples used to provide a given instar for the 3 replicates of a treatment.

IRRADIATION TREATMENT

Irradiator. Radiation treatments were conducted at the Research Irradiator in the Nuclear Institute of Tsinghua University, Beijing, China, with a 1.0×10^{15} Bq colbalt-60 source of gamma radiation. Dosimetry was done with the Fricke system according to ASTM E1026-13 (2002).

Radiation Treatment. Each of the 5 *C. sasakii* instars were irradiated within apples with a series of doses between 20 and 140 Gy, depending on the instar. Each treatment (dose) was replicated 3 times. For each test, 10 infested apples with the same instar were loaded into a plastic basket (9 × 25 × 30 cm). The baskets were placed at 100 cm around the cobalt-60 source, and irradiated with each target dose, as appropriate. To obtain uniform exposure each basket was turned 180° at mid-treatment. Fricke dosimeters were placed at the bottom, middle and top layer of the basket to monitor the absorbed doses. The measured dose rate was 2.0 Gy/min and the dose uniformity ratio was 1.18.

REARING AFTER IRRADIATION

All the irradiated and untreated apples infested with *C. sasakii* were placed in the rearing room. Each replicate was placed in a transparent plastic box (33 × 20 × 23 cm). The late fifth instars that emerged from apples were collected and weighed each d until the apples had decomposed. These larvae were transferred to moist sawdust for pupation and eclosion.

DATA ANALYSIS

For each treatment (dose) and the control, data on the number and mean weights of 5th instars that emerged from the apple fruits, and mean cumulative larval developmental times were subjected to one-way analysis of variance (ANOVA). Means were separated by Tukey's multiple comparison tests. To determine the effects of irradiation of each instar on subsequent larval development—i.e., mean cumulative larval developmental times and weights of 5th instars—the data were subjected to linear regression and analysis of covariance (ANCOVA) using the Tukey model (DPS Version13.5, Hangzhou RuiFeng Information Technology Co. Lt., Hangzhou, China).

Results

Radiation Effects on Body Weight

Body weights of *C. sasakii* late fifth instars decreased significantly in comparison to the respective controls when irradiation doses ranging from 40–100 Gy had been applied to 1st ($F = 22.5$; df = 5,12; $P < 0.0001$) and 2nd instars ($F = 48.2$; df = 5,12; $P < 0.0001$); when doses ranging from 60–100 Gy had been applied to 3rd instars ($F = 34.4$; df = 5,12; $P < 0.0001$); and when doses ranging from 60–120 Gy had been applied to 4th instars ($F = 39.9$; df = 5,12; $P < 0.0001$). In contrast, the opposite trend was observed with body weight change when 5th instars were irradiated, i.e., body weight increased significantly with increasing doses between 100–140 Gy applied to 5th instars ($F = 14.0$; df = 6,14; $P < 0.0001$) (Table 1). When 1st, 2nd, 3rd and 4th instars had been irradiated with 80 Gy, the mean weights of the 5th instars were reduced by 17.5%, 26.7%, 38.1%, and 29.3% respectively; and when these same instars had been irradiated with 100 Gy, the mean weights of the 5th instars were reduced by 25%, 29.3%, 52.5%, and 34.1%, respectively. The largest change in mean weight of the 5th instars (52.5% compared to control) took place after the 3rd instars had been irradiated with 100 Gy, the highest dose used for this stage (Table 1). The results when analyzed by ANCOVA also showed the mean weights of 5th instars varied according to which instar had been irradiated, and the irradiation dose and instar irradiated had a significant interaction ($F = 90.2$; df = 4,83; $P < 0.0001$).

Linear regression—in which weight is the dependent variable designated by y and the dose of radiation is the independent variable designated by x—was used to test whether slopes ($\Delta y/\Delta x$) were significantly different from 0 (significant effect of radiation dose). Slopes gradients—change in mean weight of the 5th instars per increment in dose of irradiation—were negative and significant for 1st, 2nd, 3rd, and 4th instars. In contrast, the gradient was positive when the 5th instars were irradiated (Table 2), indicating that the response to radiation effects on body weight in the 5th instars of *C. sasakii* was contrary to the trends observed with respect to mean weight of the 5th instars when any of the 4 for preceding instars had been irradiated.

Table 1. Effects of irradiation of each *Carposina sasakii* instar on subsequent larval development, i.e., mean number of larvae that reached the 5th instar, mean weight of late 5th instars that exited from 'Red Fuji' apples and mean cumulative larval developmental time. Each treatment was replicated 3 times.

Stage	Dose (Gy)	Mean no. of 5th instars that exited fruits and that remained in fruits	Mean weight (mg) of late 5th instars that exited the fruits	Mean cumulative larval developmental time (d)
L_1	0	56.0 ± 3.6a	24.0 ± 5.5a	26.3 ± 3.2b
(3-d)	20	46.0 ± 4.4ab	23.0 ± 5.6a	26.3 ± 4.2b
	40	40.7 ± 5.0ab	21.3 ± 5.2b	25.9 ± 4.0b
	60	33.3 ± 5.1bc	20.5 ± 4.4bc	27.1 ± 4.0b
	80	37.7 ± 6.7bc	19.8 ± 6.2cd	29.7 ± 3.5a
	100	22.7 ± 8.7c	18.0 ± 4.3d	29.9 ± 3.8a
L_2	0	44.3 ± 1.5a	23.2 ± 5.6a	26.4 ± 3.3c
(6-d)	20	31.7 ± 3.5ab	22.5 ± 4.8a	26.8 ± 4.5bc
	40	41.0 ± 3.1a	20.1 ± 5.2b	27.5 ± 4.1bc
	60	42.0 ± 5.3a	18.8 ± 4.4bc	28.8 ± 3.6a
	80	22.0 ± 3.0bc	17.0 ± 4.0cd	31.3 ± 3.4a
	100	12.7 ± 6.5c	16.4 ± 4.9d	32.7 ± 3.6a
L_3	0	33.3 ± 4.6a	24.4 ± 5.2a	25.7 ± 3.3c
(9-d)	20	25.0 ± 1.7ab	22.5 ± 5.8a	27.6 ± 4.4bc
	40	27.0 ± 12.1ab	21.0 ± 4.7ab	27.5 ± 4.1b
	60	12.3 ± 9.5bc	17.2 ± 4.4bc	29.3 ± 3.3b
	80	11.0 ± 1.0bc	15.1 ± 5.2cd	31.9 ± 4.5a
	100	5.0 ± 1.0c	11.6 ± 3.5d	31.3 ± 3.2a
L_4	0	46.3 ± 5.5a	24.6 ± 4.1a	23.2 ± 2.7c
(12-d)	40	43.0 ± 8.7ab	21.8 ± 3.3a	25.0 ± 3.0ab
	60	40.0 ± 7.5ab	18.0 ± 4.1b	26.7 ± 3.5ab
	80	34.3 ± 5.9ab	17.4 ± 4.6b	26.5 ± 3.6bc
	100	26.3 ± 6.0bc	16.2 ± 4.9b	26.5 ± 3.9ab
	120	9.0 ± 4.4c	16.1 ± 7.2b	28.0 ± 3.2a
L_5	0	35.7 ± 8.1ab	22.1 ± 4.8d	22.2 ± 2.9c
(15-d)	40	35.3 ± 4.5ab	22.6 ± 4.1d	23.2 ± 3.0bc
	60	34.0 ± 11.4ab	23.1 ± 5.1cd	23.7 ± 2.7abc
	80	41.7 ± 5.5ab	23.7 ± 5.0bcd	24.2 ± 3.2ab
	100	46.7 ± 4.7a	24.2 ± 5.4abc	24.5 ± 3.3ab
	120	24.7 ± 6.8b	24.7 ± 6.4ab	24.9 ± 3.4ab
	140	25.7 ± 5.5b	25.1 ± 6.0a	23.9 ± 3.8a

Within each larval stage, means followed by the same letter within a column are not significantly different ($P > 0.05$; Tukey's multiple comparison test); L_1: 1st instar. L_2: 2nd instar, L_3: 3rd instar, L_4: 4th instar and L_5: 5th instar

Effect of Irradiating 1 Instar at a Time on Mean Cumulative Larval Developmental Time

In general, the mean cumulative larval developmental time of *C. sasakii* irradiated during 1 of the 5 larval stadia was significantly greater than that of non-irradiated larvae. Mean cumulative larval developmental time significantly increased with increasing doses as seen when 40 Gy was applied to 2nd instars ($F = 28.3$; df = 5,12; $P < 0.0001$), 3rd instars ($F = 32.6$; df = 5,12; $P < 0.0001$), and 4th instars ($F = 9.6$; df = 5,12; $P = 0.0007$); and when 80 Gy was applied to 1st instars ($F = 32.3$; df = 5,12; $P < 0.0001$) and 5th instars ($F = 7.0$; df = 6,14; $P = 0.0001$), respectively (Table 1). Mean cumulative larval developmental time analyzed by ANCOVA also showed that the duration of development increased with increasing dose; and that the radiation dose and instar had a significant interaction ($F = 14.9$; df = 4,83; $P < 0.0001$). Linear regression was used to analyze the relationship between mean cumulative larval developmental time and radiation dose applied to each instar, but only to 1 instar at a time. Regression slopes were positive and significant for all instars (Table 3), indicating that the mean cumulative larval developmental time increased with increasing dose regardless of which instar was irradiated.

Table 2. Linear regression analyses of the mean weights of late *Carposina sasakii* 5th instar larvae with the irradiation doses when each of the instars was irradiated with doses ranging as widely as 20–140 Gy.

Stage	Obs.	y-intercept ± SE	Slope ± SE	R^2	*P value*
L_1	18	24.0 ± 0.31	−0.060 ± 0.0051	0.8953	< 0.0001
L_2	18	23.5 ± 0.30	−0.077 ± 0.0058	0.9367	< 0.0001
L_3	18	25.3 ± 0.62	−0.130 ± 0.0103	0.9088	< 0.0001
L_4	18	23.6 ± 0.65	−0.071 ± 0.0084	0.8179	< 0.0001
L_5	21	21.9 ± 0.20	0.023 ± 0.0022	0.8439	< 0.0001

L_1: 1st instar. L_2: 2nd instar, L_3: 3rd instar, L_4: 4th instar and L_5: 5th instar

Table 3. Linear regression analyses of the mean cumulative larval developmental times of the 5 *Carposina sasakii* instars irradiated with doses ranging as widely as 20–140 Gy.

Stage	Obs.	y-intercept ± SE	Slope ± SE	R^2	*P value*
L_1	18	25.5 ± 0.44	0.041 ± 0.0076	0.6405	< 0.0001
L_2	18	25.6 ± 0.41	0.065 ± 0.0067	0.8552	< 0.0001
L_3	18	25.8 ± 0.42	0.060 ± 0.0070	0.8246	< 0.0001
L_4	18	23.5 ± 0.33	0.037 ± 0.0043	0.8266	< 0.0001
L_5	21	22.5 ± 0.31	0.016 ± 0.0034	0.8144	0.0002

L_1: 1st instar. L_2: 2nd instar, L_3: 3rd instar, L_4: 4th instar and L_5: 5th instar

Mortality and Radiotolerance of Fifth Instars

All late 5th instars of *C. sasakii* were counted, including those that exited out of the apples by themselves and those that remained in the fruits; the latter being collected by hand. Table 1 shows the sum of both categories of 5th instars. The mean number of late fifth instars was significantly lower after a dose of 60 Gy had been applied to 1st instars (F = 11.3; df = 5,12; P = 0.0003), after 80 Gy had been applied to 2nd instars (F = 22.2; df = 5,12; P < 0.0001), after 60 Gy had been applied to 3rd instars (F = 8.3; df = 5,12; P = 0.0013), and after 100 Gy had been applied to 4th instars (F = 13.5; df = 5,12; P = 0.0001), indicating that irradiation induced larval mortality of either the irradiated or the subsequent instar(s). But when 5th instars were irradiated with doses ranging between 40–140 Gy, the number of survivors was not significantly less than in the non-irradiated control (F = 3.8; df = 6,14; P = 0.0181) (Table 1). This indicated that the 5th instars are more radiotolerant than the preceding instars.

Discussion

The effects of irradiation on actively developing larvae of an insect species depend on the irradiation dose and the instar or physiological condition of the insect. If irradiation prolongs larval life, it often prevents pupation so that larvae eventually die without pupating (Tilton & Brower 1983). In this study involving *C. sasakii*, significant larval mortality following irradiation occurred in the early instars (1st, 2nd and 3rd) but no significant mortality of 5th instars was observed in comparison with their controls (Table 1). This agrees with the findings of Zhan et al. (2014b) that tolerance to irradiation in *C. sasakii* increased with increasing age and developmental stage as judged by the efficacies of doses that drastically minimize the emergence of late fifth instars or adults.

Irradiation breaks chemical bonds in DNA and other molecules, thereby sublethal doses of radiation might prevent normal growth, development or reproduction of the organism (Hallman 2003, 2004; Koo et al. 2011). Moreover, ionizing radiation breaks chromosomes, and the broken chromosome ends may fuse in new combinations to form dicentric chromosomes and acentric fragments. During cell division dicentric chromosomes impede cell division and acentric fragments are lost to create genetic imbalances. These changes result in cell death (Muller 1927, 1950; LaChance 1967; Robinson 2005). In most insect species the adult stage is highly radiotolerant because dividing cells do not occur in somatic tissues but only in the gonads (LaChance 1967).

Normally, irradiated larvae have a prolonged larval stage and may live longer than non-irradiated larvae when irradiated with lower doses (Tilton & Brower 1983). Hallman (2000) noted that insects may live for considerable periods of time after irradiation. Although the developmental times of all *C. sasakii* larvae could not be determined because some larvae failed to emerge from the apples, we observed that the mean developmental time of immatures that became 5th instars that emerged from the fruits increased significantly with increasing dose as compared to the controls (Tables 1 and 3). The mean developmental time of the immatures developing in 'Red Fuji' apples in the controls of various tests (Table 1) varied between 22.2 ± 2.9 and 26.4 ± 3.3 d, which is somewhat longer than was reported in 'Golden Delicious' apples (18.13 ± 0.22 d) and much longer than the 12.48 ± 0.10 d in fruits of Japanese plum (*Prunus salicina* L.; Rosales: Rosaceae: Amygdaloideae) (Lei et al. 2012). Our results agree with those on *Ephestia kuehniella* Zeller that showed a dose-dependent delay in the developmental period of eggs and larvae. The mean cumulative larval development time of the irradiated larvae that developed to adult emergence increased compared to controls (Ayvaz & Tuncbilek 2006). This phenomenon was also noted by Wang et al. (2006) and Zhan et al. (2011) who observed that the larval development time was longer when *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae) and *Monochamus altarnatus* Hope (Coleoptera: Cerambycidae) were irradiated with 9 MeV X-ray doses of 20–60 Gy.

The larval weights of late 5th instars—developing from preceding immature stages of *C. sasakii* that had been irradiated with doses between 60–120 Gy—decreased significantly (Tables 1 and 2), suggesting that the cells associated with digestion or the enzymes related to metabolism were seriously damaged by the radiation (Boshra 2007). The results agree with other investigations that irradiation decreased the amount of food consumed and digested due to the effect of irradiation on the activity of digestive enzymes. For instance, the mean weight of 18-d old larvae of *Ephestia cautella* Walker (Lepidoptera: Pyralidae) was only 45.9% compared to the control when 1 d-old larvae were irradiated at 80 Gy (Boshra 2007). The weight of irradiated larvae of *Heliothis virescens* F. (Lepidoptera: Noctuidae) and *Sesamia cretica* (Lederer) (Lepidoptera: Noctuidae) were significantly less than normal larvae at each stage (Proshold & Bartell 1972; Abdel-Kawy & El-Naggar 1992). Increasing the dose from 20 to 60 Gy caused the larval growth rate of *Tribolium confusum* Jacquelin Du Val (Coleoptera: Tenebrionidae) to decrease from 83.0% to 38.2% of the untreated controls (Ahmed & Hassan 2001). Linear regression of body weight of *C. sasakii* showed that the mean weight of late 5th instars is correlated with radiation doses because the R^2 was ≥ 0.8179 for all stages (Table 2). The slope represents the weight reduction rate, the units of measurement being mg per Gy. When 3rd instars of *C. sasakii* were irradiated the slope (−0.13 mg/Gy) was nearly 2-fold as great as when the 1st, 2nd, and 4th instars were irradiated (−0.06 to −0.077 mg/Gy) (Table 2), indicating that weight reduction in treated 3rd instars is more severe than in these other instars. This may be caused by a greater sensitivity of the cells associated with digestion or the enzymes related to metabolism in the 3rd instar than in the other instars.

A surprising finding in this research was that the response to radiation in irradiated 5th instars of *C. sasakii* showed a trend that was opposite to other immature stages since larval weight of irradiated 5th instars significantly increased with increasing doses (Tables 1 and 2). This would indicate that the 5th instars—the last stage before pupation—differs from the preceding instars regarding feeding and excretion characteristics. Some lepidopterans adjust their life cycle to seasonal changes by arresting development in a specific phase of the last larval instar (Eizaguirrea et al. 2005). It can be postulated that a similar phenomenon occurs upon irradiation of the last larval stage.

In conclusion, gamma irradiation with doses up to 140 Gy affects the development of *C. sasakii* larvae, with significant effects on larval weight, cumulative larval developmental time, and mortality. These conclusions can be elaborated as follows:

Body Weight. The mean body weight of late 5th instars that developed from the 4 preceding instars decreased significantly in a dose-dependent manner when larvae in any one of these preceding instars had been irradiated with gamma rays. Mean body weight reduction of late fifth instars was greatest when 3rd instars were irradiated. In contrast, when 5th instars were irradiated their mean body weight increased significantly. *Cumulative Larval Development Time*. The mean cumulative larval developmental times increased significantly in a dose-dependent manner in comparison to the controls when any one of the 5 instars was irradiated. *Larval Mortality.* Significant larval mortality occurred in the first 4 instars when any one of them had been irradiated, but none of the irradiated 5th instar larvae died in the apple fruits, indicating that the 5th instar is the most radiotolerant larval stage. *Additional Research*. Further research is suggested to elucidate the mechanisms whereby the weights of 5th instar larvae that developed from irradiated 3rd instars are substantially diminished. Also research is suggested to elucidate the mechanisms whereby why the weights of 5th instars increase substantially after they have been irradiated.

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References Cited

Abdel-Kawy FK, El-Naggar SEM. 1992. Effect of gamma irradiation on the consumption digestion and utilization of food in larvae of *Sesamia cretica* (Led.). Bulletin of Entomological Society of Egypt, Economic Series 19: 203-209.

Ahmed ZA, Hassan NMM. 2001. Effect of four rearing diets and gamma irradiation on larval growth rate and the development of the confused flour beetle *Tribolium confusum*(Duv.). Arab Journal of Nuclear Sciences and Applications 34: 315-322.

ASTM E1026-13. 2002. Standard practice for using the Fricke dosimetry system. West Conshohocken, PA, USA.

Ayvaz A, Tuncbilek AS. 2006. Effects of gamma radiation on life stages of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Journal of Pest Science 79: 215-222.

Bell C. 2000. Fumigation in the 21st century. Crop Protection 19: 563-569.

Boshra SA. 2007. Effect of gamma irradiation of food consumption, assimilation and digestive enzymes in *Ephestia cautella* (Walker) larvae. Journal of Stored Products Research 43: 49-52.

DPS (Data Processing System). 2010. User's guide. Version 13.5. Hangzhou Rui-Feng Information Technology Co., Ltd. Hangzhou, China.

EPPO. 1997. Data Sheets on Quarantine Pests, CABI and EPPO. Quarantine pests for Europe: data sheets on quarantine pests for the European Union and for the European and Mediterranean Plant Protection Organization. Wallingford: CAB International in association with the European and Mediterranean Plant Protection Organization.

Eizaguirrea M, Schafellner C, Carmen L, Sehnal F. 2005. Relationship between an increase of juvenile hormone titer in early instars and the induction of diapause in fully grown larvae of *Sesamia nonagrioides*. Journal of Insect Physiology 51: 1127-1134.

Hallman GJ. 2000. Expanding radiation quarantine treatments beyond fruit flies. Agricultural and Forest Entomology 2: 85-95.

Hallman GJ. 2003. Ionizing irradiation quarantine treatment against plum curculio (Coleoptera: Curculionidae). Journal of Economic Entomology 96(5): 1399-1404.

Hallman GJ. 2004. Ionizing irradiation quarantine treatment against Oriental fruit moth (Lepidoptera: Tortricidae) in ambient and hypoxic atmospheres. Journal of Economic Entomology 97(3): 824-827.

Hallman GJ. 2011. Phytosanitary applications of irradiation. Comprehensive Reviews in Food Science and Safety 10(2): 143-151.

Hua BZ. 1992. On the scientific name of the peach fruit borer. Entomotaxonomia 14(4): 313-314.

Huang KX, Wu WJ. 1975. Peach fruit moth. Entomological Knowledge 4: 34-41.

Koo HN, Yoon SH, Shin YH, Yoon C, Woo JS, Kim GH. 2011. Effect of electron beam irradiation on developmental stages of *Plutella xylostella* (Lepidoptera: Plutellidae). Journal of Asia-Pacific Entomology 14: 243-247.

LaChance LE. 1967. The induction of dominant lethal mutations by ionizing radiation and chemicals as related to the sterile-male technique of insect control, pp. 617-650 *In* Wright JW, Pal R. [eds.], Genetics of Insect Vectors of Disease. Elsevier, Amsterdam, The Netherlands.

Lei X, Li D, Li Z, Zalom FG, Gao L, Shen Z. 2012. Effect of host plants on developmental time and life table parameters of *Carposina sasakii* (Lepidoptera: Carposinidae) under laboratory conditions. Environmental Entomology 41(2): 349-354.

Li DX, Lei XH, Li Z, Gao LW, Shen ZR. 2012a. Effects of different host plants on the development and reproduction of the peach fruit borer, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae). Acta Entomologica Sinica 55(5): 554-560.

Li L, Liu T, Zhang FH, Li TX, Wang LJ, Wang YJ. 2012b. Research on the toxicity of methyl bromide against hawthorn spider mite and peach fruit moth. Plant Quarantine 26(1): 6-9.

Liu B, Zhang FH, Wang YJ. 2010. Toxicity of phosphine to *Carposina niponensis* (Lepidoptera: Carposinidae) at low temperature. Journal of Economic Entomology 103(6): 1988-1993.

Muller HJ. 1927. Artificial transmutation of the gene. Science 66: 84-88.

Muller HJ. 1950. Radiation damage to genetic material. American Scientist 38: 33-59, 126.

Proshold FD, Bartell JA. 1972. Post embryonic growth and development of F_1 and F_2 tobacco budworm from partially sterile males. Canadian Entomologist 104: 165-172.

Robinson AS. 2005. Genetic basis of the sterile insect technique, pp. 95-114 *In* V. A. Dyck, J. Hendrichs, and A. S. Robinson [eds.], Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. Springer, Dordrecht, The Netherlands.

Ryu J, Ahn JY, Lee SS, Lee JW, Lee KY. 2015. Developmental inhibition of gamma irradiation on the peach fruit moth *Carposina sasakii* (Lepidopetra: Carposinidae). Radiation Physics and Chemistry 106: 136-139

Tilton EW, Brower JH. 1983. Radiation Effects of Arthropods, pp. 270-277 *In* Josephson ES, Peterson MS [eds.], Preservation of Food by Ionizing Radiation. Volume II, Florida: CRC Press, Inc.

Wang YJ, Wang X, Zhan GP, Xu L, Liu ZJ, Liu B, Sun ZC, Zhe CM, Huang XX. 2006. Effect of irradiation on development of the larvae of *Anoplophora glabripennis*. Journal of Nuclear Agricultural Sciences 20(6): 527-530.

Zhan GP, Wang X, Wan YJ, Hu M, Li BS, Yang SJ, Huang M. 2011. Investigation on the irradiation of *Monochamus alternates* by 9 MeV X-rays. Plant Quarantine 25(4): 12-17.

Zhan GP, Li Y, Li TX, Wang YJ, Li BS, Qin HL. 2013. The primary investigation on the development of the eggs of peach fruit moth after irradiation by Cobalt-60 γ-rays. Plant Quarantine 27(1): 51-55.

Zhan GP, Gao MX, Ye BH, Li BS, Li TX, Wang YJ, Liu B, Qin HL. 2014a. Irradiation effect on the eclosion rate of *Carposina sasakii* mature larvae. Journal of Nuclear Agricultural Sciences 28(3): 453-458.

Zhan GP, Li BS, Gao MX, Liu B, Wang YJ, Liu T, Ren LL. 2014b. Phytosanitary irradiation of peach fruit moth (Lepidoptera: Carposinidae) in apple fruits. Radiation Physics and Chemistry 103: 153-157.

Zhan GP, Ren LL, Gong SR, Li TX, Li BS, Wang YJ. 2014c. A comparison study on the radiation tolerance of *Carposina sasakii* (Lepidoptera: Carposinidae) pupae. Plant Quarantine 28(5): 27-31.

Zhang HQ, Wang YL. 1983. The inherited sterility of peach fruit borer (*Carposina nipponensis* Wais.) resulting from irradiation. Application of Atomic Energy in Agriculture 4: 1-10.