



# Effect of $^{60}\text{Co}$ - $\gamma$ Ray Radiation on the Reproduction of Cochineal (Homoptera: Coecoidea: Dactylopiidae)

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**Abstract:**  $^{60}\text{Co}$ - $\gamma$  ray has been used to develop new varieties of cochineal. The purpose of this study was to assess the effect of  $^{60}\text{Co}$ - $\gamma$  ray radiation on the reproduction of cochineal. Different doses of  $^{60}\text{Co}$ - $\gamma$  ray were used to treat cochineal and the ability of generating offspring was determined. The results showed that high dose of  $^{60}\text{Co}$ - $\gamma$  ray treatment dramatically reduced the ability of cochineal to produce offspring.  $F_1$  generation of cochineal treated with high dose of  $^{60}\text{Co}$ - $\gamma$  (3000rad) only produced small number of  $F_2$  generation and  $F_2$  cochineal were no longer able to produce offspring. In contrast, low dose of  $^{60}\text{Co}$ - $\gamma$  ray treatment dramatically increased the number of female  $F_1$ , while the number of male  $F_1$  was only slightly increased compared with those untreated cochineal. Under the dose of 500rad, the reproductive ability of  $F_2$  generation was significantly increased, but the female body volume and the number of brooding eggs were relatively small. Under the dose of 1000rad, the female body volume and the number of brooding eggs were not significantly different from those untreated cochineal. The female body volume and the number of brooding eggs in  $F_3$  generation were slightly higher than the control under both 500rad and 1000rad doses.

**Keywords:**  $^{60}\text{Co}$ - $\gamma$  Ray, Radiation, Cochineal, Offspring, Biological Characteristics

## 1. Introduction

Radiation, a recently developed breeding method, has the advantages of high mutation frequency, stability of the inherited character, and rapidness of generating new genotypic alterations. Radiation, especially  $\gamma$ -ray radiation, has been used in both plant and animal breeding. In the study of insects,  $\gamma$ -ray has been used for more than 150 insect species, but mainly for the cultivation of sterile insects to achieve the purpose of pest control [9, 13, 8, 7, 12]. The use of  $\gamma$ -ray to breed resource insects has rarely been reported. Previous studies have examined the mutation and breeding of *Trichogramma* by  $^{60}\text{Co}$ - $\gamma$  radiation of embryo, larva, pre-pupa, pupa and adult stages at five different doses [2, 11], showed that low-dose irradiation of *Trichogramma* at egg and late pupa stages had no influences on their viability. Irradiated of *Trichogramma* at late pupa stage increased the female/male ratio at  $F_1$  generation, but had no influence on the following three consecutive generations. Chen et al. [3], used  $^{60}\text{Co}$ - $\gamma$  to

treat China *Kerria* and lac *Kuwana* and found that treatment of lac eggs embryo at developmental 2-3 stages could produce offspring. Treatment of lac *Kuwana* at embryonation 5-6 stages with 11000rad doses could occasionally produce offspring. The number of brooding eggs and secreted gum of treated Lac *Kuwana* were similar to those untreated control. The effective fecundity and female sex ratio were higher than control. The solid insect density in the treated insects was lower than the control. Egg hatching rate of silkworm was significantly reduced and the mortality rate was significantly increased by  $^{60}\text{Co}$ - $\gamma$  treatment. When the radiation dose was increased to 300Gy, the silkworm egg hatching rate was reduced to zero. The development of silkworm was also reduced with the increases of the radiation doses. The lethal effect of the radiation was more profound to female silkworm than the male silkworm [5]. Tang et al. [10], showed that treatment of *Tussah* with a dose <120Gy did not have significant impact on the hatchability and mortality. However, with the increases of the dose, hatchability and mortality were significantly decreased. In addition, proteomic and genomic

changes were also observed in the blood after radiation, suggesting that  $^{60}\text{Co}$ - $\gamma$  treatment has important impact on the development of Tussah. Dai *et al.* [5], treated female pupae (2-3 day before feathering of silkworm) with  $^{60}\text{Co}$ - $\gamma$  and then mated with untreated male moths to produce offspring. In  $F_3$  generation, offspring had a new translucent silkworm variety with highly translucent skin, but without lethality. After several generations, this new translucent silkworm has strong constitution and normal fecundity. Previous studies have also shown that larval weight was increased by 21.96%, silk gland was increased by 30.14%, cocoon weight was increased by 11.11%, cocoon shell weight was increased by 9.76%, silk length and weight were increased by 22.96% and 22.53%, respectively, after treatment of the silkworm with  $^{60}\text{Co}$ - $\gamma$  at the dose of 1 Gy [1]. Radiation had minor effect on the fecundity of adult silkworm.

Cochineal (*Dactylopius coccus*) is a class of valuable resource insect that parasitizes on cactus and produce natural red pigment. The female adult body contains 19-24% Carmines acid, which can be used to produce cochineal red pigment. Improving the yield and quality of female adult worms is critical for cochineal industry [4]. In this study, we explored the possibility of using  $^{60}\text{Co}$ - $\gamma$  radiation to breed excellent variant of cochineal with high yield and quality.

## 2. Material and Methods

*Opuntia ficus-indica* Mill was used as plant for the growth of *D. coccus* Costa. The resource of radiation  $^{60}\text{Co}$ - $\gamma$  was provided by Kunming Hui Technology Development Co.

Female cochineal at spawning stage was treated with different doses of  $^{60}\text{Co}$ - $\gamma$  ray. The radiated female adults were inoculated on *D. coccus* Costa. Untreated cochineal was used as control. Biological characteristics were recorded for three

generations of cochineal ( $F_1$ ,  $F_2$  and  $F_3$ ).

Culturing of  $F_1$  generation:

A total of 420 spawning female adults were divided into 5 groups (each group contained 40 adults) treated with 500, 1000, 3000, 5000 and 8000rad  $^{60}\text{Co}$ - $\gamma$  ray, respectively. A total of 220 adults were included in the control group and were not treated with  $^{60}\text{Co}$ - $\gamma$  ray. Each experimental group was inoculated on 2 pots of cactus and the control group was inoculated on 11 pots of cactus.

Culturing of  $F_2$  generation:

After complete maturation,  $F_1$  generation was remained on the plant for natural proliferation. In the 8000rad group, 2 and 5 adults were remained in each pot. In the 5000rad group, 12 and 12 adults were remained in each pot. In the 3000rad group, 21 and 12 adults were remained in each pot. In the 1000rad group, 17 and 50 adults were remained in each pot. In the 500rad group, 48 and 78 adults were remained in each group. In the control group, 30 adults were remained in each plant.

Culturing of  $F_3$  generation:

After complete maturation,  $F_2$  adults were collected (10 adults/plant).  $F_1$  generation from 8000rad, 5000rad and 3000rad groups were infertile, so there were no  $F_2$  offspring. Therefore,  $F_3$  generation was only cultured from 1000rad and 500rad groups.

Data analysis:

Data were processed and analyzed by Excel 2003 and SPSS 20.0.

## 3. Results

*The parameters of adults from each group*

The parameters of adults from each group were shown in Table 1, 2 and 3.

Table 1. Parameters of  $F_1$  generation.

Radiation dose (rad)	The number of adult/plant			Size of female adult (mm,mm <sup>3</sup> )				Number of eggs/female adult
	Female	Male	Total	Length	Width	Height	Volume	
0(CK)	36.2	21.5	57.7	5.867	4.391	3.471	47.257	164.3
500	78.5	25.5	104	5.870	4.536	3.399	47.646	288.3
1000	61	17	78	5.790	4.467	3.242	44.244	183.3
3000	31	0	31	5.870	4.319	3.468	46.105	166.7
5000	16	1	17	5.745	4.283	3.368	43.410	110.0
8000	7.5	0	7.5	5.640	4.223	3.263	40.741	115.5

Table 2. Parameters of  $F_2$  generation.

Radiation dose (rad)	The number of adult/plant			Size of female adult (mm,mm <sup>3</sup> )				Number of eggs/female adult
	Female	Male	Total	Length	Width	Height	Volume	
0(CK)	165.4	143.1	308.5	5.670	3.876	3.363	39.649	265.917
500	385.5	522	907.5	4.996	3.120	2.903	23.751	137.75
1000	88	170	258	5.795	3.846	3.276	38.929	279.667
3000	0	0	0	/	/	/	/	/
5000	0	0	0	/	/	/	/	/
8000	0	0	0	/	/	/	/	/

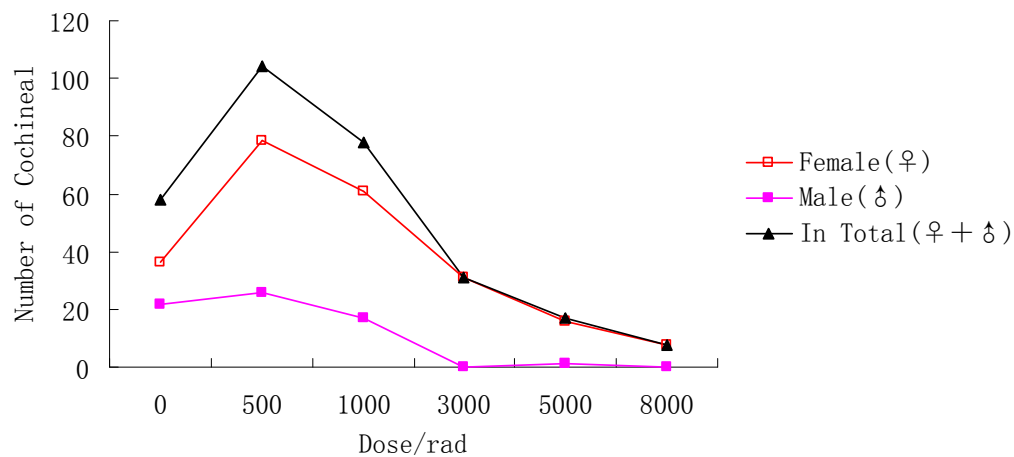
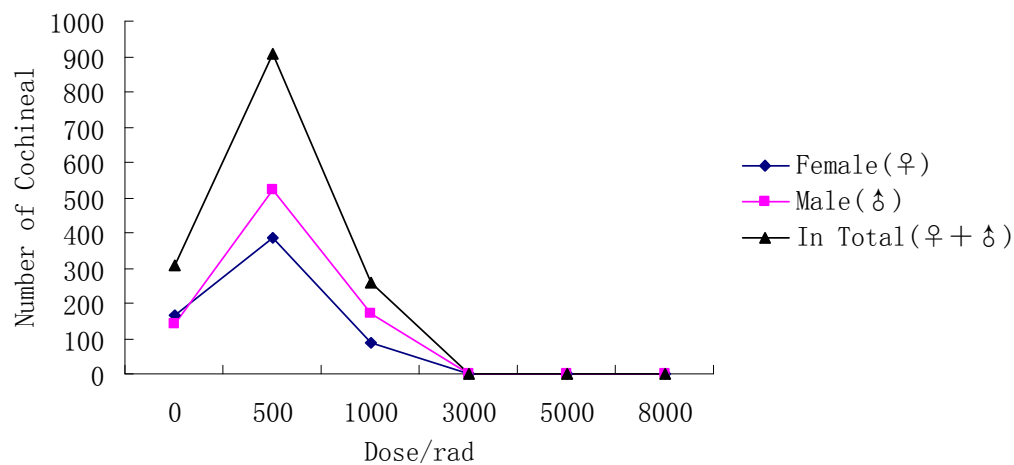
Table 3. Parameters of  $F_3$  generation.

Radiation dose (rad)	The number of adult/plant			Size of female adult (mm,mm <sup>3</sup> )				Number of eggs/female adult
	Female	Male	Total	Length	Width	Height	Volume	
0(CK)	257	181	438	5.741	4.613	3.188	44.392	327.333
500	249	121	370	5.877	4.667	3.200	46.234	363.800
1000	285	224	509	6.029	4.619	3.386	49.535	366.133
3000	0	0	0	/	/	/	/	/
5000	0	0	0	/	/	/	/	/
8000	0	0	0	/	/	/	/	/

#### *The effect of radiation on the fecundity of cochineal*

The number of  $F_1$  adults was decreased with the increases of the  $^{60}\text{Co}$ - $\gamma$  ray doses (Fig. 1). The number of  $F_2$  adults in the 500rad group was significantly higher than that in the control group (Fig. 2). However, the number of  $F_2$  adults in 1000rad group was slightly lower than that in the control group (Fig. 2). Adults of  $F_2$  generation were not observed in 3000, 5000 and 8000rad groups because female  $F_1$  was not produced in these groups. These results suggested that treatment with  $^{60}\text{Co}$ - $\gamma$  ray at a dose of being higher than 3000rad had dramatic impact on fecundity of cochineal. The number of female  $F_3$  adults in 500rad and 1000rad groups was similar to that in the control group (Fig. 3).

8000rad groups because female  $F_1$  was not produced in these groups. These results suggested that treatment with  $^{60}\text{Co}$ - $\gamma$  ray at a dose of being higher than 3000rad had dramatic impact on fecundity of cochineal. The number of female  $F_3$  adults in 500rad and 1000rad groups was similar to that in the control group (Fig. 3).

Fig. 1. The number of Cochineal in  $F_1$  adult treated with different radiation doses.Fig. 2. The number of Cochineal in  $F_2$  adult treated with different radiation doses.

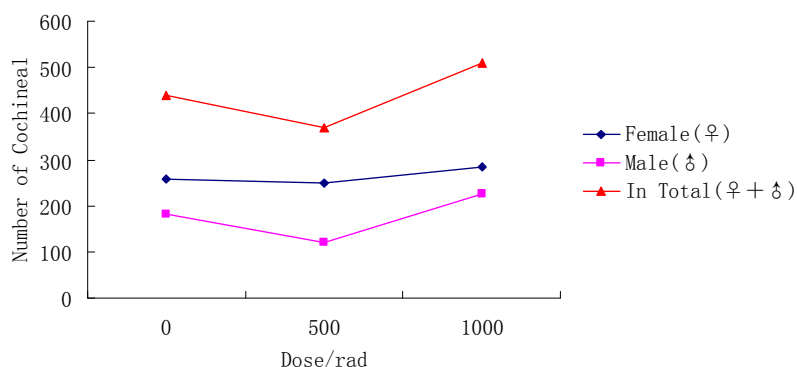


Fig. 3. The number of Cochineal in F<sub>3</sub> adult treated with different radiation doses.

#### *The effect of radiation on the body volume of adult cochineal*

The length, width, height and volume F<sub>1</sub> adult were decreased with the increases of the  $^{60}\text{Co}$ - $\gamma$  ray doses (Table 1, Fig. 4). The biggest body volume of F<sub>1</sub> adults was observed in the control and 500rad groups. The body volume of F<sub>2</sub> adults was decreased in 500rad group and then increased in 1000rad

group (Fig. 4), while the body volume of F<sub>3</sub> adults was slightly increased with the increases of radiation doses (Fig. 4). These results suggested that low dose of radiation had minor effect on the offspring volume. Radiation at the dose of 1000rad slightly increased the body volume of F<sub>3</sub> adult, but overall, in the three consecutive generations, female body volume changed dramatically without strong regularity.

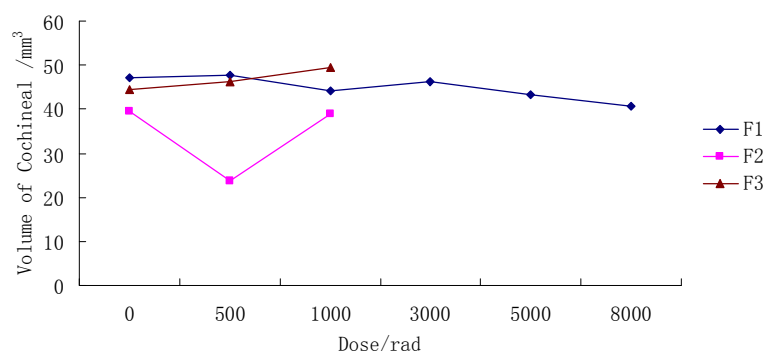


Fig. 4. The volume of an adult female in each generation treated with different radiation doses.

#### *The effect of radiation on the number of brooding eggs*

The number of brooding eggs in F<sub>1</sub> generation was slightly increased in the 500rad group (288 eggs) and then decreased with the increases of radiation doses. In contrast, the number of brooding eggs in F<sub>2</sub> generation was dramatically decreased in the 500rad group (137.8 eggs) and then increased with the

increases of the radiation doses (Fig. 5). The number of F<sub>3</sub> brooding eggs in control, 500rad, and 1000rad groups was 327.3, 363.8 and 366.1 ( $P > 0.05$ , Table 4). These results suggested that low dose of radiation can significantly increase the number of brooding eggs in F<sub>1</sub> generation, but this increase cannot be stably inherited by the following generations.

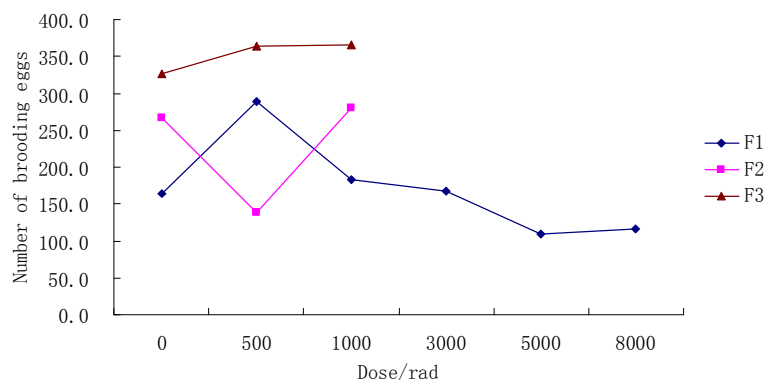


Fig. 5. The number of brooding eggs of an adult female in each generation treated with different radiation doses.

**Table 4.** Multiple comparisons on the number of brooding eggs of an adult female in each generation at different radiation doses.

	(I) dose	(J) dose	Mean Difference (I-J)	Std. Error	P value
F <sub>1</sub> generation	0	500	-64.000	32.186	0.054
		1000	-19.000	32.186	0.558
		3000	-2.400	32.186	0.941
		5000	54.300	55.747	0.336
		8000	48.800	55.747	0.387
F <sub>2</sub> generation	0	500	128.167**	33.380	0.001
		1000	-13.750	33.380	0.683
		500	-36.467	25.341	0.158
F <sub>3</sub> generation	0	1000	-38.800	25.341	0.133

## 4. Discussion

In this study, we explored the possibility of using  $^{60}\text{Co}$ - $\gamma$  ray to breed new variants of resource cochineal. Our results showed that high dose of  $^{60}\text{Co}$ - $\gamma$  ray radiation leads to severe imbalance of male and female offspring (no male offspring and thus no sexual reproduction). These results confirmed previous findings that the hatching rate of silkworm was decreased and the mortality was increased with the increases of the radiation dose [6, 10]. Because the egg sex in female adults had already formed before radiation, we concluded that high dose of  $^{60}\text{Co}$ - $\gamma$  ray treatment had dramatic damages to males. These results are different from previous studies showing more significant mortality to female silkworm than male silkworms [6], suggesting that the effect of high dose radiation was different to different species of insects.

Low dose of  $^{60}\text{Co}$ - $\gamma$  ray radiation, especially at the dose of 500rad, significantly increased the number of female adults and the number of brooding eggs in F<sub>1</sub> generation, while number of male adults was only slightly increased. After three generations, the volume and the number of brooding eggs at the dose of 500rad and 1000rad were slightly increased comparing to the control group, suggesting the possibility of breeding new variants of cochineal by low dose of  $^{60}\text{Co}$ - $\gamma$  ray radiation. However, the phenotype in the consecutive three generations was not very regular, consisting with the results of “The stimulation effect of low doses  $^{60}\text{Co}$  gamma irradiation on viability of *Trichogramma Chilonis*” [11] and “The radiation experiment for lac insects” [3]. This could be due to inconsistency of the female maturity and the small genotypic changes which was restored to wild-type after multiple generations.

Future studies will be directed to the radiation of cochineal at different instars and self recovery of the damaged gene function after radiation.

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