
Effect of ^{60}Co - γ Ray Radiation on the Reproduction of Cochineal (Homoptera: Coecoidea: Dactylopiidae)

Chen Hai-You¹, Qin Zhi-Hong², Zhang Zhong-He^{1,*}

¹Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming, China

²Xishan Forestry Farm, Kunming Forestry Bureau, Kunming, China

Email address:

664506179@qq.com (Chen Hai-You), 984767892@qq.com (Qin Zhi-Hong), Zhzhang.caf@163.com (Zhang Zhong-He)

*Corresponding author

To cite this article:

Chen Hai-You, Qin Zhi-Hong, Zhang Zhong-He. Effect of ^{60}Co - γ Ray Radiation on the Reproduction of Cochineal (Homoptera: Coecoidea: Dactylopiidae). *Agriculture, Forestry and Fisheries*. Vol. 5, No. 6, 2016, pp. 243-248. doi: 10.11648/j.aff.20160506.15

Received: October 26, 2016; **Accepted:** November 7, 2016; **Published:** December 2, 2016

Abstract: ^{60}Co - γ ray has been used to develop new varieties of cochineal. The purpose of this study was to assess the effect of ^{60}Co - γ ray radiation on the reproduction of cochineal. Different doses of ^{60}Co - γ ray were used to treat cochineal and the ability of generating offspring was determined. The results showed that high dose of ^{60}Co - γ ray treatment dramatically reduced the ability of cochineal to produce offspring. F_1 generation of cochineal treated with high dose of ^{60}Co - γ (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}Co - γ 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}Co - γ 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 γ -ray radiation, has been used in both plant and animal breeding. In the study of insects, γ -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 γ -ray to breed resource insects has rarely been reported. Previous studies have examined the mutation and breeding of *Trichogramma* by ^{60}Co - γ 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}Co - γ 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}Co - γ 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}Co - γ 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}Co - γ 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}Co - γ 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}Co - γ 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}Co - γ was provided by Kunming Hui Technology Development Co.

Female cochineal at spawning stage was treated with different doses of ^{60}Co - γ 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}Co - γ ray, respectively. A total of 220 adults were included in the control group and were not treated with ^{60}Co - γ 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 ³)				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 ³)				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 F₃ generation.

Radiation dose (rad)	The number of adult/plant			Size of female adult (mm,mm ³)				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 F₁ adults was decreased with the increases of the ⁶⁰Co-γ ray doses (Fig. 1). The number of F₂ adults in the 500rad group was significantly higher than that in the control group (Fig. 2). However, the number of F₂ adults in 1000rad group was slightly lower than that in the control group (Fig. 2). Adults of F₂ generation were not observed in 3000, 5000 and

8000rad groups because female F₁ was not produced in these groups. These results suggested that treatment with ⁶⁰Co-γ ray at a dose of being higher than 3000rad had dramatic impact on fecundity of cochineal. The number of female F₃ adults in 500rad and 1000rad groups was similar to that in the control group (Fig. 3).

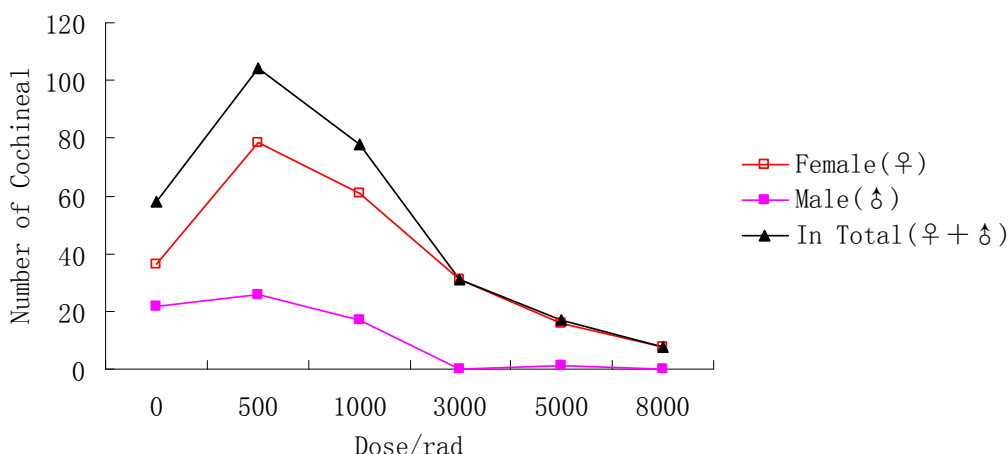


Fig. 1. The number of Cochineal in F₁ adult treated with different radiation doses.

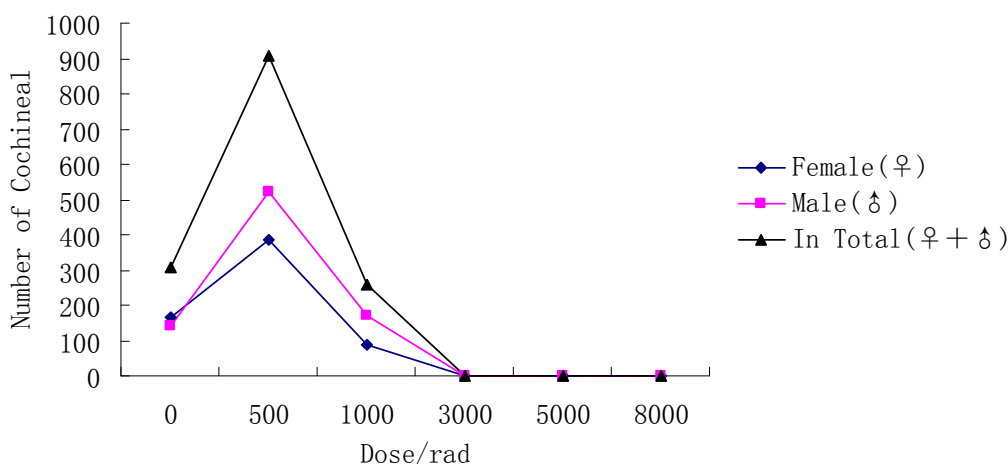


Fig. 2. The number of Cochineal in F₂ adult treated with different radiation doses.

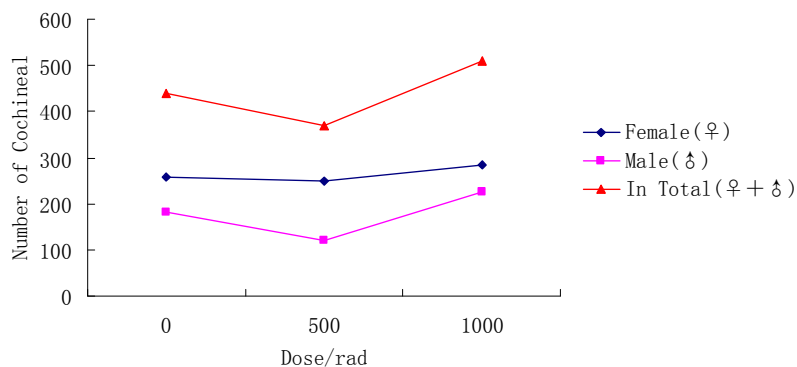


Fig. 3. The number of Cochineal in F₃ adult treated with different radiation doses.

The effect of radiation on the body volume of adult cochineal

The length, width, height and volume F₁ adult were decreased with the increases of the ^{60}Co - γ ray doses (Table 1, Fig. 4). The biggest body volume of F₁ adults was observed in the control and 500rad groups. The body volume of F₂ adults was decreased in 500rad group and then increased in 1000rad

group (Fig. 4), while the body volume of F₃ 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 F₃ adult, but overall, in the three consecutive generations, female body volume changed dramatically without strong regularity.

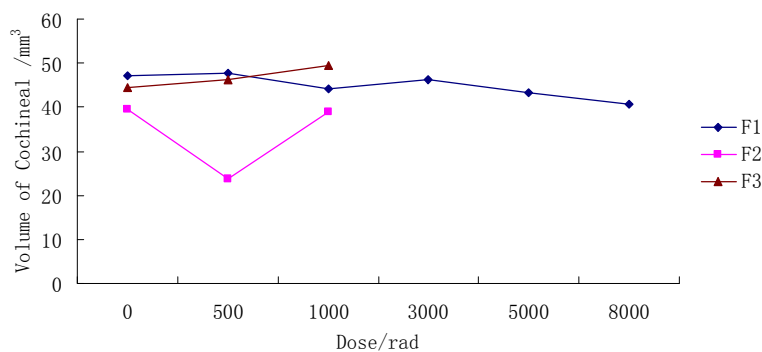


Fig. 4. The volume of an adult female in each generation treated with different radiation doses.

The effect of radiation on the number of brooding eggs

The number of brooding eggs in F₁ 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 F₂ 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 F₃ 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 F₁ generation, but this increase cannot be stably inherited by the following generations.

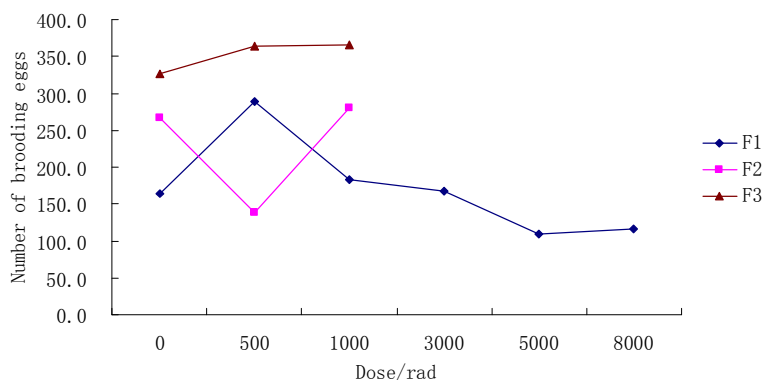


Fig. 5. The number of brooding eggs of an adult female in each generation treated with different radiation doses.

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 ₁ 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 ₂ generation	0	500	128.167**	33.380	0.001
		1000	-13.750	33.380	0.683
		500	-36.467	25.341	0.158
F ₃ generation	0	1000	-38.800	25.341	0.133

4. Discussion

In this study, we explored the possibility of using ⁶⁰Co-γ ray to breed new variants of resource cochineal. Our results showed that high dose of ⁶⁰Co-γ 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 ⁶⁰Co-γ 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 ⁶⁰Co-γ ray radiation, especially at the dose of 500rad, significantly increased the number of female adults and the number of brooding eggs in F₁ 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 ⁶⁰Co-γ 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 60Co 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.

Acknowledgements

Funds for research were provided by the Special Public Welfare of Chinese Forestry Industry Research (no. 201204602), the Special Funds of Basic Scientific Research Cost of Central Public Welfare Scientific Research Institutes (no. RIRICAF201005M) and the Chinese State Forestry Administration Spark Project (no. 2005EA169004). The Kunming Hui Technology Development Co. are thanked for

providing the resource of radiation ⁶⁰Co-γ. The authors also thank Professor CHEN XIAOMING and Professor SHI LEI, Dr. WU HAIXIA, Senior experimentalist YE SHOUBE(The Research Institute of Resource Insects, Chinese Academy of Forestry, China) for their valuable help.

References

- [1] ADBEL-SALAM A. KAMELIA & MAHMOUD M. SOUAD [1995]: Stimulating effects of low levels of ⁶⁰Co gamma ray on the silkworm, *bombyx mori* (L.).-Journal of Pest Science 68 (7): 147-150.
- [2] CHEN YUAN-LIN, ZHAO WAN-YUAN [1983]: The radiation experiment for trichogramma.-Entomological knowledge (4): 157-160.
- [3] CHEN XIAO-MING, CHEN YONG, WANG SHAO-YUN, MAO YU-FENG, FENG YING, YE SHOU-DE [1998]: The radiation experiment for lac insects.-Forest Research 11 (4): 382-387.
- [4] CHEN HAI-YOU, ZHANG ZHONG-HE, YE SHOU-DE, YANG GUANG-CAN [2013]: The cultivation situation of cochineal in the world.-Journal of southwest university, natural science edition 35 (S1): 16-20.
- [5] DAI FANG-YIN, LU CHENG, WANG XIA, XIA QING-YOU, ZHONG-HUAI XIANG [2002]: Study of genetics of ⁶⁰Co-γ-ray-induced translucent silkworm (*Bombyx Mori* L.).-Journal of southwest agricultural university 24 (2): 173-175.
- [6] DAI JUN-JUN, FAN TAO, ZHANG YU-PING, WANG CHU-YAN, WU CHUAN-HUA, LIU JIAN [2012]: Biological effects induced by high-doses ⁶⁰Co-γ radiation in silkworm, *bombyx mori*.-North Sericulture 33 (1): 9-12.
- [7] HE LI-HUA, WEN HONG-BIAO, NIU BAO-LONG, SHEN WEI-FENG, LIU YAN, MENG ZHI-QI [2009]: Influence of ⁶⁰Co-γ irradiation on the fecundity of *Dendrolimus punctatus*.-Forest Pest and Disease Journal 28(1): 25-26.
- [8] MA PEI-PEI, ZHANG YONG-AN, WANG DENG-YUAN, WANG YU-ZHU, QU LIANG-JIAN, JIANG PING, MOU JIAN-JUN [2008]: Influence of ⁶⁰Co-γ irradiation on fertility of Japanese pine sawyer beetle *Monochamus alternatus* houpe (Coleoptera: Cerambycidae).-Journal of nuclear agricultural sciences 22(1): 101-104.
- [9] SUCKLING D. M. [2003]: Applying the sterile insect technique for biosecurity: benefits and constraints.-New Zealand Plant Protection 56: 21-26.

- [10] TANG LIANG-WEN, LIU CHAO-LIANG, ZHU BAO-JIAN, WEI GUO-QING, WANG ZAI-GUI, SUN HUI, YAO LI-HU, WU XIANG, ZHOU YAN, QIAN CEN, CAO JIA [2009]: The research on biological characteristics of *Antheraea pernyi* radiated by ^{60}Co - γ ray.-Acta laser biology sinica 18 (6): 740-745.
- [11] WANG EN-DONG, LI YONG-JUN, LIU XIAO-HUI, LU DA-GUANG [2004]: The stimulation effect of low doses ^{60}Co gamma irradiation on viability of *Trichogramma Chilonis*.-Acta Agriculturae Nucleatae Sinica 18 (5): 406-410.
- [12] ZHONG GUO-HUA, CHEN YONG, YANG HONG-XIA, HU MEI-YING [2012]: Advances in application and research of sterile insect irradiation techniques.-Plant Protection 38 (2): 12-17.
- [13] ZHOU LI-JUAN, HU MEI-YING, XU HAN-HONG, HUANG JI-GUANG, ZHONG GUO-HUANG [2004]: Recent studies on the physiological effect of irradiation on insects.-Acta Agriculturae Nucleatae Sinica 18(1): 77-80.