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Effects of Electron Beam Irradiation on Comstock Mealybug, *Pseudococcus comstocki* (KUWANA) (Homoptera: Pseudococcidae)

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Abstract : Eggs, larvae and mated mature females of comstock mealybugs, *Pseudococcus comstocki* (KUWANA) were irradiated with electron beams in order to determine their radiosensitivities. When the eggs were irradiated, younger eggs (0~5 day-old and 6~10 day-old) were more sensitive than older eggs (11 day-old and 12 day-old). The larvae irradiated at 0.2 kGy did not grow to maturity. The adult females irradiated at 0.2~0.6 kGy oviposited, but their eggs did not hatch except at 0.2 kGy. The descendants, which hatched from eggs irradiated at 0.2 kGy, grew more slowly than the control, and the female descendants still maintained their fecundity.

Key words : radiation, Pseudococcidae, *Pseudococcus comstocki*, sterility, hatchability

Introduction

Arthropod pests which are found on cut flowers imported into Japan are mostly thrips, mites, Lepidoptera and Hemiptera. About 20% of imported cut flowers are disinfested with chemical fumigants such as methyl bromide and cyanic acid. However, the use of methyl bromide will be restricted or limited in the near future, because of the environmental problem, in which this chemical substance is regarded as a breaker of the ozone zone. The development of alternative treatments is keenly required. Preliminary research has indicated that irradiation can be a disinfestation method as a quarantine procedure (WIT and VRIE, 1985; GOODWIN and WELLHAM, 1990; YATHOM, *et al.*, 1990, 1991; ELBADRY, *et al.*, 1972). But there are few studies which have dealt with irradiation of mealybugs.

In this report, the authors investigated the effects of electron beam irradiation on eggs, larvae and adult females of comstock mealybug, *Pseudococcus comstocki*.

Materials and Methods

1. Rearing

Test insects were obtained from a laboratory colony of comstock mealybug, which were reared in polyethylene containers (14cm in hight, 18cm in diameter) at 23±1℃ and 70% r.h., under a photoperiod of 24D. They were reared on pumpkin, *Cucurbita moschata* (DUCHESNE) (DUCHESNE ex POIR). The developmental duration of the eggs was 12 days under these rearing conditions. Adult males emerged in about 30 days and adult females laid eggs in about 50 days after hatching.

2. Eggs

The eggs were divided into four groups (0～5 day-old, 6～10 day-old, 11 day-old and 12 day-old eggs) for the exposure to electron beams. Zero～5 day-old and 6～10 day-old eggs were obtained from one female which was allowed to oviposit for 5 days in a petri dish under the rearing conditions. Eleven day-old and 12 day-old eggs were obtained from 6 females allowed to oviposit for 24 hours. These eggs were stored under the rearing conditions until they were tested. In 12 day-old eggs, hatched larvae were removed immediately after irradiation. Irradiated eggs were held under the rearing conditions and the hatchability was recorded.

3. Larvae

The larvae obtained from the eggs which were stored for 13 days under the rearing conditions were irradiated. After irradiation, 150 larvae (50 larvae × 3 replications) were inoculated on a pumpkin in each treatment. Subsequent development of them was observed.

4. Adult females

Five adult females were irradiated in a petri dish. The survival rate and ovipositional ability of irradiated adult females were determined for the duration of 14 days after irradiation at 3 or 4 day intervals. The hatchability was also calculated.

Fifteen irradiated adult females (5 adult females × 3 replications) were inoculated on a pumpkin and removed after 14 days so that the subsequent development and fecundity of their descendants could be observed.

5. Irradiation

The mealybugs were irradiated with electron beams under conditions of 20℃ and 50～60%r.h. in a Van de Graaff electron accelerator (Nissin High Voltage Co. Ltd., 2.5 MeV, 1.5×10^6 Gy/hr). The absorbed dose was controlled by changing the beam current at a conveyer speed of 3.0 m/min. Beam currents were 29.8, 59.6 and 89.7 μA for 0.2, 0.4 and 0.6 kGy, respectively. Doses were measured with a radiochromic film (RCF) dosimeter (FWT-60-00, Far West Technology Inc.) (HAYASHI, *et al.*, 1992). All tests were replicated three times.

Results

1. Eggs

Younger eggs (0～5 day-old and 6～10 day-old) were more sensitive to electron beams than older eggs (11 day-old and 12 day-old) (Table 1). The dose-hatchability relations of the 11 day-old and the 12 day-old eggs are expressed by the following regression lines.

11 day-old eggs; $y = 86.31-133.20x$ ($r = -0.9986$)

12 day-old eggs; $y = 91.08-105.35x$ ($r = -0.9979$)

The calculated LD_{50} of 11 day-old and 12 day-old eggs were 0.2726 kGy and 0.3898 kGy, respectively. Noteworthy was the irradiation tolerance which increased abruptly between 10 day-old eggs and 11 day-old eggs.

Table 1. Effects of irradiation on hatchability at the different ages of irradiated *P.comstocki* eggs

Dose (kGy)	Hatchability (%)			
	0~5 day-old eggs	6~10 day-old eggs	11 day-old eggs	12 day-old eggs
0	91.6±1.6 (287)[1]	89.3±2.3 (1145)	84.6±2.1 (1556)	91.3± 5.6(1131)
0.2	0 (316)	0.1±0.1 (1271)	61.2±7.8 (1466)	71.0±10.4(1230)
0.4	–	0 (1342)	34.9±8.1 (1549)	46.7± 7.8(1088)
0.6	–	–	4.6±2.9 (1424)	29.1± 8.5(966)

1) Value in parenthesis is a sum of the tested eggs in three replications.

Table 2. Effects of irradiation on *P.comstocki* larvae

Dose (kGy)	The number of stages 40 days after irradiation				
	Tested[1] larvae		Adults		
		Dead larvae	Males	Females	Eggs laid[2]
0	150	0	128	22	10,000~
0.2	150	150	0	0	–
0.4	150	150	0	0	–
0.6	150	150	0	0	–

1) One hundred and fifty neonate larvae (50 larvae × 3 replications) were used for each level.
2) Sum of the eggs which were yielded by females.

2. Larvae

The effects of irradiation on larvae were shown in Table 2. All of the irradiated larvae died off during immature stages and none grew to the adult.

3. Adult females

Table 3 shows the effects of irradiation on adult females. The survival rates of irradiated and non-irradiated adult females were almost the same for a week after irradiation. But thereafter, the survival rate of the control declined more than that of treated individuals. The ovipositional abilities of irradiated individuals were reduced and were finally depressed to less than a half of that of the control 4 days after the irradiation. Although 2.2% of total eggs from irradiated adult females at 0.2 kGy hatched 0~4 day after irradiation, other eggs which were exposed to dose higher than 0.2 kGy did not hatch. Survivors developed slowly and adults of both sexes were obtained (Table 4).

Discussion

As eggs grew older, the irradiation tolerance increased (Table 1). It is clear that there is a large shift of tolerance between 10 day-old and 11 day-old eggs. Similar shifts were also

Table 3. Effects of irradiation on adult females of *P. comstocki*

Dose (kGy)	Tested females[1]	Days after irradiation	Mortality (%)	Ovipositional ability (eggs/♀/day)	Hatchability (%)
0	15	0～ 4	0	57.8	87.8±1.6
		4～ 7	6.7	25.1	86.4±6.3
		7～11	20.0	6.6	99.2±0.9
		11～14	66.7	1.2	100
0.2	15	0～ 4	0	36.7	2.5±2.3
		4～ 7	0	11.4	0
		7～11	26.7	2.1	0
		11～14	40.0	0.4	0
0.4	15	0～ 4	0	41.5	0
		4～ 7	6.7	8.6	0
		7～11	20.0	0.9	0
		11～14	40.0	0.1	0
0.6	15	0～ 4	0	35.3	0
		4～ 7	6.7	9.2	0
		7～11	20.0	0.6	0
		11～14	33.0	0	-

1) Fifteen mature adult females (5 females × 3 replications) were used for each level.

Table 4. Effects to the next generation[1] of irradiated adult females of *P.comstocki*

Dose (kGy)	Tested[2] females	Eggs laid[3]	Descendants[4]			
				Adults		
			Larvae	Males	Females[5]	Eggs laid[6]
0	15	5,000～	34	165	73 (25)	10,000～
0.2	15	1,836	0	1	5 (0)	0
0.4	15	2,022	0	0	0	-
0.6	15	1,528	0	0	0	-

1) Used the identification method of stages (MURAKAMI, 1964).
2) Fifteen mature adult females (5 females × 3 replications) were used for each level.
3) Sum of the eggs which were yielded by females 2 weeks after irradiation.
4) The constructions of descendant were researched 60 days after irradiation.
5) Value in parenthesis is the number of female descendant which oviposited.
6) Sum of the eggs which were yielded by adult female descendants.

observed for the codling moth, *Cydia pomonella* and two spotted spider mite, *Tetranychus urticae* (TOBA and BURDITT, 1992; DOHINO and TANABE, 1993) during the development of their eggs periods. The change of tolerance in aged eggs was synchronized with the embryogenesis or the embryonic development, in which the former was in the red ring stage and the latter had developed extremities. So it is supposed that, from the 10 day-old to 11 day-old eggs of *P.comstocki*,

some embryological developments might occur and that they might be closely related to the irradiation tolerance. Actually, under the microscope, formed extremities were observed in that period.

The radiosensitivity was slightly different between the eggs before and after oviposition. Zero~5 day-old eggs irradiated at 0.2 kGy did not hatch but a few eggs from the adult females irradiated at the same dose did hatch (Table 1, 3, 4). The change of tolerance was also present in two spotted spider mite (DOHINO and TANABE, 1993). It is known that there is a variable tolerance of gonial cells in spermatogenesis or in oogensis and that cleavage or segmentation is more sensitive to irradiation (SZLENDAK *et al.*, 1992). The changes of tolerance of eggs in *P.comstocki* and *T.urticae* seem to be explained by their results.

Neonate larvae irradiated at 0.2 kGy or higher, were killed at early stage (Table 2). The decrease in larval radiosensitivity in developing larval instar was reported in various arthropod pests such as american serpentine leafminer, *Liriomyza trifolii*, codling moth, *Cydia pomonella*, two spotted spider mite, *Tetranychus arabicus* and *T.urticae* (YATHOM, *et al.*, 1990; BURDITT and HUNGATE, 1989; ELBADRY *et al.*, 1972; DOHINO and TANABE, 1994), and further studies with developing larvae of *P.comstocki* will reveal the details of the larval radiosensitivity.

A few descendants from adult females which had been irradiated at 0.2kGy, survived and grew to the adult stage. Finally, one male descendant and 5 female descendants emerged (Table 3, 4). One of the females laid a few sterile eggs. It is considered that these eggs were produced by one of the females which successfully mated with the male in that colony. They needed about 10 more days for oviposition than the time required for the control. Five female descendants were crossed with non-irradiated males in order to investigate their fecundities. All of the females yielded more viable eggs. The cross test indicated that the female descendants were not sterilized yet but the male descendant was. Therefore, it suggests that the adult females irradiated at 0.2 kGy can maintain their fecundities.

In disinfestation of *P.comstocki* with electron beam irradiation, doses of 0.4 kGy or higher were required in our experiments, although further studies are needed both with the subsequent development of individuals from the most tolerant eggs and with developing larvae. Our experiment suggests that electron beam irradiation can be used as a quarantine procedure against *P.comstocki*.

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和 文 摘 要

クワコナカイガラムシ *Pseudococcus comstocki* (KUWANA) (Homoptera: Pseudococcidae) の電子線照射

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クワコナカイガラムシ *Pseudococcus comstocki* の卵，幼虫及び雌成虫に0.2，0.4，0.6kGyの電子線（2.5MeV）をそれぞれ照射し，その影響を調べた。

1) 若齢卵（0～5日齢卵，6～10日齢卵）は老熟卵（11日齢卵，12日齢卵）よりも感受性が高かった。

2) 幼虫は0.2kGy以上の照射で完全に殺虫された。

3) 雌成虫を照射した結果，0.2kGy区では次世代が得られ，成虫にまで達した。妊性調査により，雄成虫は不妊化されていたが，雌成虫では妊性が認められた。0.4kGy区以上では次世代が得られなかった。