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Effects of Electron Beam Irradiation on Eggs and Larvae of *Spodoptera litura* (FABRICIUS) (Lepidoptera : Noctuidae)

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Abstract : Eggs (1-4-day-old), 3rd instar larvae and 5th instar larvae of tobacco cutworm, *Spodoptera litura* were irradiated with electron beams (2.5 MeV). Eggs varied their tolerance against irradiation with embryonic development. Third instars were more radiosensitive than 5th instars. Although the irradiation could not prevent the 5th instars feeding even at 400Gy, their adult emergence was inhibited at 100Gy or higher. There was a conspicuous tendency toward weight loss of 3rd instars due to the reduction of their food consumption, while such a tendency was not shown clearly for 5th instars.
Key words : radiation, *Spodoptera litura*, hatchability, food consumption, commodity treatment

Introduction

A total number of cut flowers including cut foliage imported into Japan in 1994 reached 808 million stalks. Of that amount 12.7% of imported cut flowers were disinfested by methyl bromide or hydrogen cyanide fumigation due to insect and arthropod pests such as thrips (34.1% of pests intercepted in quarantine inspection), mites (18.0%), scales and mealybugs (13.8%), Lepidoptera (13.0%), aphids (11.4%) and other pests (9.7%) (Yokohama Plant Protection Station, MAFF, 1995).

Methyl bromide as a fumigant is regarded as an ozone depleting substance and the use of methyl bromide will be restricted or limited in the near future (TATEYA, 1993). We, therefore, tested the effect of electron beam irradiation against thrips, mites, mealybugs and leafminers as one alternative to methyl bromide fumigation (DOHINO and TANABE, 1994 ; DOHINO and MASAKI, 1995 ; KUMAGAI and DOHINO, 1995 ; DOHINO *et al.*, 1996).

Tobacco cutworm, *Spodopeta litura,* is one of the serious polyphagous pests of domestic horticulture. There are some useful reports about the effects of gamma irradiation on this pest (KIYOKU and TSUKUDA, 1969 ; WAKID and HAYO, 1974 ; SOUKA, 1980 ; SETH and SEHGAL, 1986, 1987a, 1987b) and their reports, however, concern lower or sub-lethal doses of gamma irradiation for sterile insect release technique, not for quarantine. Thereupon we studied the susceptibility of eggs and larvae to electron beam irradiation, and the effect of the irradiation on larval feeding behaviour, because there are many of these insect stages found on cut flowers at import inspection.

Materials and Methods

Test insects

S. litura were obtained from Yokohama Research Center, Mitsubishi Chemical Corporation. Seven or eight adult pairs were allowed to oviposit on the inside of a tracing paper bag (21 × 30 × 18 cm) at 25 ± 1 ℃ and 60-80% r.h., under a photoperiod of 16L : 8D. The eggs were put in a petri dish and held under the conditions as mentioned above for preparing 1, 2, 3- and 4-day-old eggs until irradiation. Larvae were given an artificial diet (Insecta LF ®, Nihon Nosan Kogyo K.K.) in a plastic container (21 × 28 × 10 cm). Third instar larvae (3rd instars were obtained 6-7 days after hatching) and fifth instar larvae (5th instars mixed with 2.8% fourth instars were obtained 12-13 days after hatching) were prepared for irradiation. Newspaper straps were spread in the container for their pupation. Pupae were obtained about 20 days after hatching.

Irradiation and the evaluation for insects

Approximately 300 of each aged egg stage (1, 2, 3- and 4-day-old) on tracing paper in a petri dish were irradiated with electron beams. Similar experiments were performed to observe the subsequent developments of survivors. This test was replicated three times. Twenty of each larval stage with the artificial diet in a polyethylene cup (4cm in height and 10cm in diameter) were irradiated, and the test was replicated five times.

The electron beam irradiation was carried out with a Van de Graaff electron accelerator (Nissin High Voltage Co. Ltd., 2.5 MeV, 1.5 × 10^6 Gy/hr) at National Food Research Institute, Tsukuba, Ibaraki. The accelerator was operated at 20 ℃ and 50-60% r.h. The absorbed dose was controlled by changing the beam current at a conveyer speed of 3.0m/min. Beam currents were 14.7, 29.4 and 58.8 μA for 100, 200 and 400Gy, respectively. The doses were measured with a radiochromic film (RCF) dosimeter (FWT-60-00, Far West Technology Inc.) (HAYASHI *et al.*, 1992). After irradiation, egg stages were held under the above mentioned conditions and counted a number of hatched larvae 7 days after irradiation under the microscope. Hatched larvae were kept in storage to observe their subsequent developments. Larval stages were also held under the same conditions, where not only their mortality, but also their food consumption was recorded. The food consumption (g/larva/day) was determined by the following formula. After measuring the weight, a new diet was prepared for the next measuring.

Food consumption = B − (Ab/a)

A ; weight of artificial diet at start of food consumption test (control or uninfested).
a ; weight of artificial diet 1 day after food consumption test started (control or uninfested).
B ; weight of artificial diet at the start of food consumption test (unirradiated and irradiated larvae).
b ; weight of artificial diet 1 day after food consumption test started (unirradiated and irradiated larvae).

Results and Discussion

Susceptibility of eggs to irradiation

Susceptility of egg stage (1, 2, 3- and 4-day-old) to irradiation is shown in Fig.1. One-day-old eggs, 2-day-old eggs and 3-day-old eggs were killed completely at 100Gy, 100Gy and 400Gy, respectively, while 35.9% of 4-day-old eggs at 400Gy hatched. The data showed clearly that 4-day-old eggs were the most tolerant of all egg stages. By the regression analysis, 713Gy was required to prevent the hatching of 4-day-old eggs. SETH and SEHGAL (1989) reported that older eggs were more tolerant than younger eggs in susceptibility of *S. litura* eggs to gamma irradiation. TOBA and BURDITT (1992) reported that the irradiation tolerance of eggs of codling moth, *Cydia pomonella*, related to embryogenesis and that 5-6-day-old eggs (blackhead stage) were more tolerant than younger egg stages (newly laid egg and red ring

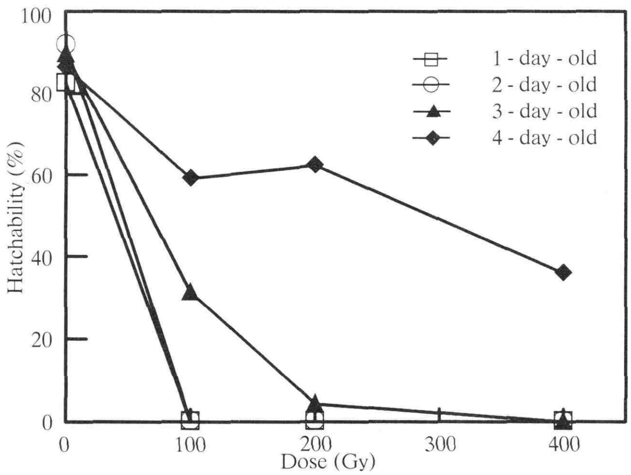

Fig. 1. Hatchability of different ages of *S.litura* eggs irradiated with electron beams.

stage). In their report there was a change in tolerance to irradiation at 3-4-day-old eggs (the red ring stage). According to the phenology model suggested by RICHARDSON *et al.* (1982), the red ring stage was in the embryogenic process in which head capsules and cervical shields were not heavily sclerotized. A change in tolerance was observed in 3-day-old eggs in our experiment. Under the microscope, 3-day-old eggs were in the stage in which head capsules were not sclerotized, while 4-day-old eggs were in the stage in which head capsules were clearly distinguishable from the rest of embryo. The effect of irradiation differed with egg age. Treated 1-day-old eggs almost died before head capsule sclerotizing, while the head capsules of unhatched 3-day-old eggs were melanized, and median response was shown in 2-day-old eggs.

Subsequent developments of the hatched larvae are shown in Fig.2. Survivors died in the first instars at 400Gy. No survivors pupated at 200Gy. Some survivors attained pupae, however, no adults emerged at 100Gy.

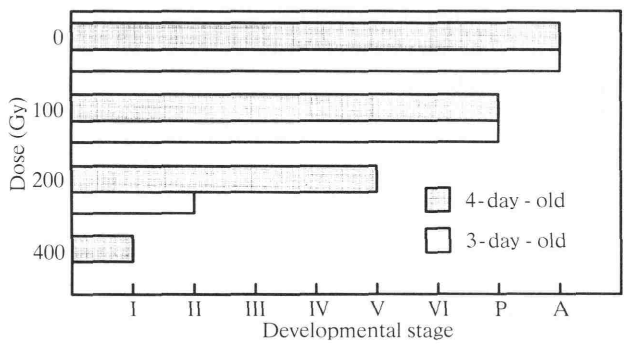

Fig. 2. Subsequent development of *S.litura* eggs irradiated with electron beams.
I - VI ; larval stages (1st-6th instar),
P ; pupa, A ; adult

Table 1. Effects of electron beam irradiation on 3rd and 5th instar larvae of *S.litura*

Age[1]	Dose (Gy)	No. of larvae[2]	No. of larvae III	IV	V	VI	No.of pupae Abnormal	Normal	No.of adults (♂:♀)
3rd	0	100	0	0	0	5	0	95	83 (34:49)
	100	100	34	44	13	6	3	0	0 (−)
	200	100	87	9	4	−	−	−	−
	400	100	92	8	−	−	−	−	−
5th	0	100	−	0	0	4	0	96	86 (34:52)
	100	100	−	0	9	44	47	0	0 (−)
	200	100	−	0	11	82	7	0	0 (−)
	400	100	−	0	9	91	−	−	−

1) Tested 5th instars mixed with 2.8% 4th instars.

2) Total number of larvae in 5 replicates.

Table 2. Food consumption and larval weight of *S.litura* larvae irradiated with electron beams

Dose (Gy)	3rd instar larva[1] Food consumption	Weight	5th instar larva[2] Food consumption	Weight
0	0.296 g/day	1.247 g	0.263 g/day	1.228 g
100	0.046	0.704	0.247	0.865
200	0.010	0.048	0.225	0.774
400	0.001	0.023	0.175	0.824

1) Food consumption continued to be measured for 14 days and larval weight was recorded 14 days after irradiation.

2) Food consumption continued to be measured for 7 days and larval weight was recorded 7 days after irradiation.

Susceptibility of larvae to irradiation

The result of the larval stage experiment is shown in Table 1. Both 3rd instars and 5th instars (including 2.8% 4th instars) could not emerge at 100Gy or higher. Irradiated 3rd instars became swollen and blackened in the same manner as *S. egigua* larvae irradiated by gamma ray (WIT and van de VRIE, 1985). The mortality in the earlier stage increased in proportion to the increase of dosage.

Older larvae continued their larval metamorphoses but they failed in pupation or became malformed pupae much like the malformed pupae which had survived in experiments with gamma irradiation (WIT and van de VRIE, 1985 ; SETH and SEHGAL, 1987a). It is considered that irradiation worked strongly on larval ecdysis at 3rd instars, and on pupation at 5th instars.

SETH and SEHGAL (1987a) reported that 6th instars of *S. litura* were more tolerant than 3rd instars ; *i. e.* pupation of irradiated younger larvae with gamma ray at 170Gy was prevented and they died before the penultimate larval moult at 240Gy, while some older larvae achieved pupation at 200Gy, but all died in prepupae at 280Gy. Their results on irradiated 3rd instars were almost identical to ours. Therefore, if the experiment of the most tolerant 6th instars is done with electron beams, the response of the 6th instars to irradiation should be almost identical to SETH and SEHGAL's result, based on our data of irradiated 5th instars.

Effects of irradiation on food consumption and weight of larvae

The food consumption of 3rd instars and 5th instars was in inverse proportion to the rise in dosage as shown in Table 2. Especially, irradiation strongly depressed the feedings of 3rd instars. At 400Gy the feedings were inhibited within several days, however, the feedings continued for 2 weeks at 100Gy. On the other hand, irradiation could not inhibit the feedings of 5th instars, although food consumption of 5th instars irradiated at 400Gy was reduced in two-thirds of unirradiated larvae. Relations of dosage-food consumption for 5th instars were expressed by the following regression line; $y = 0.2666 - 0.0002x$ ($r = -0.9960$). From this expression, 1,333Gy was required to inhibit their feeding behaviour. After irradiation, a 5th instar larva irradiated at 400Gy will eat 6.250 cm^2 of chrysanthemun leaf or 2.692 cm^2 of carnation leaf in a day. One square centimeter of the leaf of chrysanthemum and carnation corresponded to 0.028g and 0.065g, respectively. The feeding will deteriorate the quality of the ornamentals. There was a conspicuous tendency for 3rd instars to lose weight due to the reduction of their food consumption, while such a tendency was not clear for 5th instars.

Our data indicate that 400Gy irradiation with electron beam inhibited the hatching, larval growth and the pupation of *S. litura* eggs and larvae. Also, the data indicate that the 400Gy treatment could not prevent the feedings of 5th instars although the treatment contributed to the reduction of the larval feedings. The survivors irradiated at 400Gy could not produce offspring, and the treatment will prevent this pest from contaminating imported cut flowers and domestic horticulture. Irradiation at 400Gy does not cause severe damage or injury to many varieties of cut flowers (WIT and van de VRIE, 1985 ; TANABE and DOHINO, 1993, 1995). It is, therefore, considered that electron beam irradiation is a potential quarantine treatment to disinfest *S. litura* eggs and larvae.

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和 文 摘 要

ハスモンヨトウ *Spodoptera litura* (FABRICIUS)の卵及び幼虫に対する電子線照射の効果

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ハスモンヨトウ *Spodoptera litura* の卵及び幼虫 (3齢幼虫及び4～5齢幼虫) に100Gy, 200Gy及び400Gyの電子線(2.5MeV)を照射し、その影響を調べた。

1) 卵では日齢により照射耐性が変化し、胚発生が進んだ卵ほど耐性が高かった。最も耐性の高かった4日齢卵を400Gyで照射したとき、その孵化幼虫は、2齢まで達しなかった。
2) 3齢幼虫よりも4～5齢幼虫の方が照射耐性が高かった。4～5齢幼虫の摂食は、400Gyでも阻止することはできなかったが、対照区と比較して3分の2の摂食量に抑制できた。また、100Gy以上の照射で羽化を阻止できた。