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Electron Beam Irradiation of Eggs and Adult Females of Two Spotted Spider Mite, *Tetranychus urticae* KOCH (Acari: Tetranychidae)

Toshiyuki DOHINO and Kazuo TANABE

Physical Control Laboratory, Research Division, Yokohama Plant Protection Station, Kitanaka-dori 6-64, Naka-ku, Yokohama 231, Japan

Abstract: Aged eggs and mated mature females of *Tetranychus urticae* were exposed to electron beams (2.5MeV). The tolerance of eggs increased with age (1～5-day-old). Five-day-old eggs irradiated at 0.4kGy and 0.6kGy developed into adults, but only adult females were completely sterilized. Females irradiated at 0.4kGy or higher did not produce viable eggs, although temporary recovery of fecundity was observed at 0.2kGy.

Key words: Arachnida, *Tetranychus urticae*, radiation, sterility, hatchability

Introduction

The number of cut flowers imported into Japan in 1991 was 435 millions, of which 22.1% was infested with pests, e.g. thrips (39.3%), mites (19.4%), Lepidoptera (12.0%), aphids (11.2%), and other pests (18.1%). These pests represent a potential danger to domestic agriculture.

Tetranychus mites are difficult pests to control, since they acquire resistances to chemicals, and exhibit a wide variety of responses to acaricides (SMITH and FULTON, 1951; DENNEHY *et al.*, 1987; GRAFTON-CARDWELL *et al.*, 1987). Especially, problematic is the two spotted spider mite *Tetranychus urticae* KOCH, since they live and cause economic damage to a wide variety of horticultural plants throughout the year. Application of ionizing radiation to the control of *T. urticae* infesting ornamental plants has been investigated to develop quarantine treatment alternative to fumigation (GOODWIN and WELLHAM, 1990; HENNEBERRY, 1964; TANABE and KATO, 1992; WIT and VRIE, 1985).

In the present study, the effects of electron beam irradiation on development of *T. urticae* eggs and on the fecundity of females were investigated to estimate sterile doses required in a quarantine treatment.

Materials and methods

Rearing

Two spotted spider mites were obtained from the Faculty of Horticulture, Chiba University. Mites were reared with kidney bean leaves at 22℃ and 70% r.h. under photoperiod of 16L : 8D. Under these conditions, the duration of eggs, and larval and nymphal stages were 4～7 days and 7～9 days, respectively. And adult females were

obtained about 9 days after hatching.

Eggs

Five mated females were allowed to oviposit on a bean leaf which was put on 0.6% agar medium including 20ppm crystal violet according to MATSUNAGA and FURUHASHI (1972) with the modifications shown in Fig. 1. After 24 hours the females were removed and the number of eggs laid on leaves was recorded. Eggs were held under rearing conditions until irradiation. In the case of 5-day-old eggs, any larvae observed immediately after irradiation were removed from bean leaf. After irradiation, hatchability and emergence rate at each stage were recorded. Afterwards, adult emergence pairs of irradiated and non-irradiated mite were separately reared to determine their fecundity. When non-irradiated mite died, new ones were supplied.

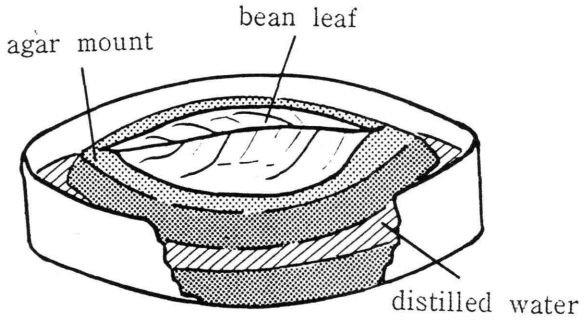

Fig. 1. Leaf disk method
Kidney bean leaf was put on agar mount upside down. Distilled water was supplied to prevent mites escaping and agar drying.

Females

Bean leaves infested with mite coloy including mated mature females, which were in the status after first oviposition, were irradiated. After irradiation, five females were transferred to a new bean leaf on the agar and held under rearing condition. Mites were transferred to new leaf every two or three days and the number of eggs laid on leaves was recorded. Hatchability of these eggs and survivors were observed until adult emergence.

Irradiation

Mites were irradiated in a Van de Graaff electron accelerator (Nissin High Voltage Co. Ltd., 2.5MeV, 1.5×10^6 Gy/hr) at National Food Research Institute, Kannondai 2-1-2, 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 3.0 m/min. The absorbed dose was determined with the cellulose triacetate (CTA) dosimeter (Model FTR-125, Fuji Photo Film Co. Ltd.) (HAYASHI, 1992). Beam currents were 29.8 μA, 59.6 μA, 89.7 μA, 119.2 μA, 149.0 μA and 149.0 + 59.6 μA for 0.2kGy, 0.4kGy, 0.6kGy, 0.8kGy, 1.0kGy and 1.4kGy, respectively. After irradiation, eggs were held under rearing conditions.

All tests were replicated five times.

Results

Eggs

T. urticae eggs varied its tolerance against irradiation with embryonic development (Table 1). At the doses tested, the tolerance remarkably increased 3 days after oviposition, when eye-spots were observed. Dose-hatchability relationship in old eggs gave regression as follows; 3-day-old eggs, $y=33.513-42.000x$ ($r=-0.847$); 4-day-old eggs, $y=111.486-113.770x$ ($r=-0.964$); 5-day-old eggs, $y=112.575-41.404x$ ($r=-0.826$), where LD_{50} in hatchability of 4-day-old eggs and 5-day-old eggs were

Table 1. Effects of irradiation on hatchability in different ages of *T. urticae* eggs[1)]

Dose (kGy)	1-day-old	2-day-old	3-day-old	4-day-old	5-day-old
0.2	0 (230)[2)]	0 (155)	25.2±5.9 (190)	85.9± 1.1 (155)	−
0.4	0 (211)	0 (135)	16.6±2.6 (188)	70.7±13.0 (155)	96.2± 4.2 (181)
0.6	0 (231)	0 (141)	8.4±5.4 (196)	45.5±12.3 (159)	85.6± 6.6 (163)
0.8	0 (218)	0 (146)	−	13.1± 6.3 (165)	82.0± 8.2 (188)
1.0	0 (218)	0 (151)	−	0.9± 1.2 (160)	71.2±14.4 (182)
1.4	−	−	−	−	54.0±15.6 (171)

1) Mated mature females were allowed to oviposit on kidney bean leaves for 24 hours. Five females per leaf were used. Hatchability of non-irradiated control was 98.1±2.0% (n=199).
2) Value in parenthesis is a sum of tested eggs in five replications.

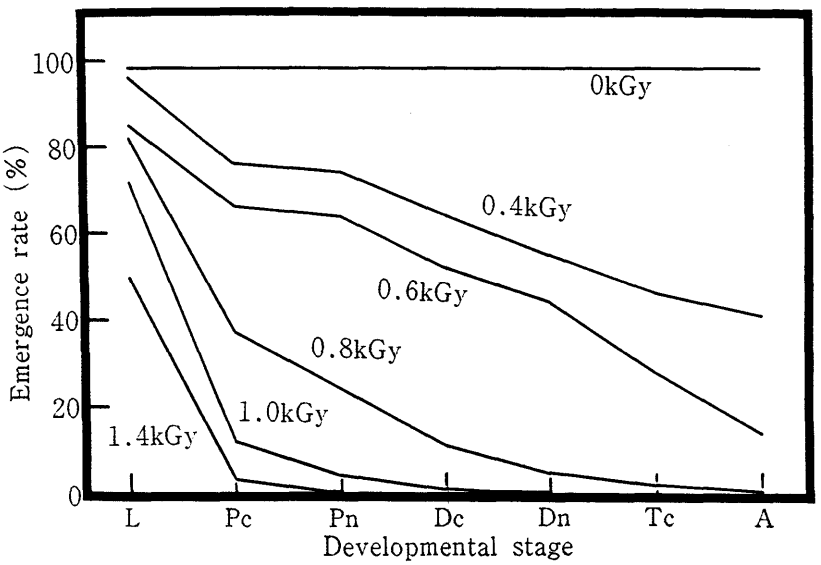

Fig. 2. Emergence rate of immature stages from irradiated 5-day-old eggs
L; larva, Pc; protochrysalis, Pn; protonymph
Dc; deutochrysalis, Dn; deutonymph,
Tc; teleiochrysalis, A; adult

Table 2. Effects of irradiation on development and fecundity in 5-day-old eggs of *T. urticae*

Dose (kGy)	Tested[1] eggs	Mean of immature[2] duration (days)	Adults[3] emergence	Sex ratio (♀ : ♂)	Ovipositional rate of progeny (eggs/♀/day)
0	199	12.4	195 (98.0)	3.4 : 1	7.0
0.4	181	15.3	74 (40.9)	100 : 0	0
0.6	163	17.2	23 (14.1)	100 : 0	0
0.8	188	—	0 (0.0)	—	—

1) Value is a sum of five replications.
2) Value includes egg duration.
3) Value in parenthesis is percentage of adult emergence.

0.5kGy and 1.3kGy, respectively. Five-day-old eggs were the most tolerant and there was no significant difference in hatchability between control and eggs irradiated at 0.4kGy. However, irradiation reduced emergence rate in subsequent stages (Fig. 2). In 5-day-old eggs irradiated at 0.8kGy or higher, the metamorphosis of larva to protochrysalis was strongly inhibited and no adults emerged. Development of immature stages was delayed by irradiation (Table 2). The sex ratio of adults from non-irradiated eggs was ♀ : ♂=3.4 : 1, but all adult from eggs irradiated at 0.4kGy or higher were female and did not oviposit. These females were inactive and could hardly feed. They showed indistinct 'two spots' and white-yellow bodies in contrast with dark green in non-irradiated control. Malformation on 4th legs, which lacked segments and twisted, was noticed in adults from irradiated 3- and 4-day-old eggs but 5-day-old eggs.

Females

Irradiated females changed body color 3 days after irradiation similar to that from irradiated eggs. The survival rate of irradiated females slowly decreased 5 days after irradiation, while that of non-irradiated females rapidly decreased 7 days after irradiation and was lower than that of irradiated females 9～10 days after irradiation (Fig. 3-A). The inequality of the survival rate in irradiated females was reversed 12 days after irradiation. Oviposition of irradiated females rapidly declined in first 7 days and stopped 17, 10, 7 and 7 days after irradiation at 0.2, 0.4, 0.6 and 0.8kGy, respectively (Fig. 3-B). Eggs from females irradiated at 0.4kGy or higher did not hatch out. At 0.2kGy, hatchability recovered from 0.6～2.1% in the first 7 days up to 28.5～46.2% in the next 7days, although the ovipositional rate continued decreasing during both periods (Fig. 3-B and -C). Survivors from eggs irradiated at 0.2kGy developed into adults (males and females).

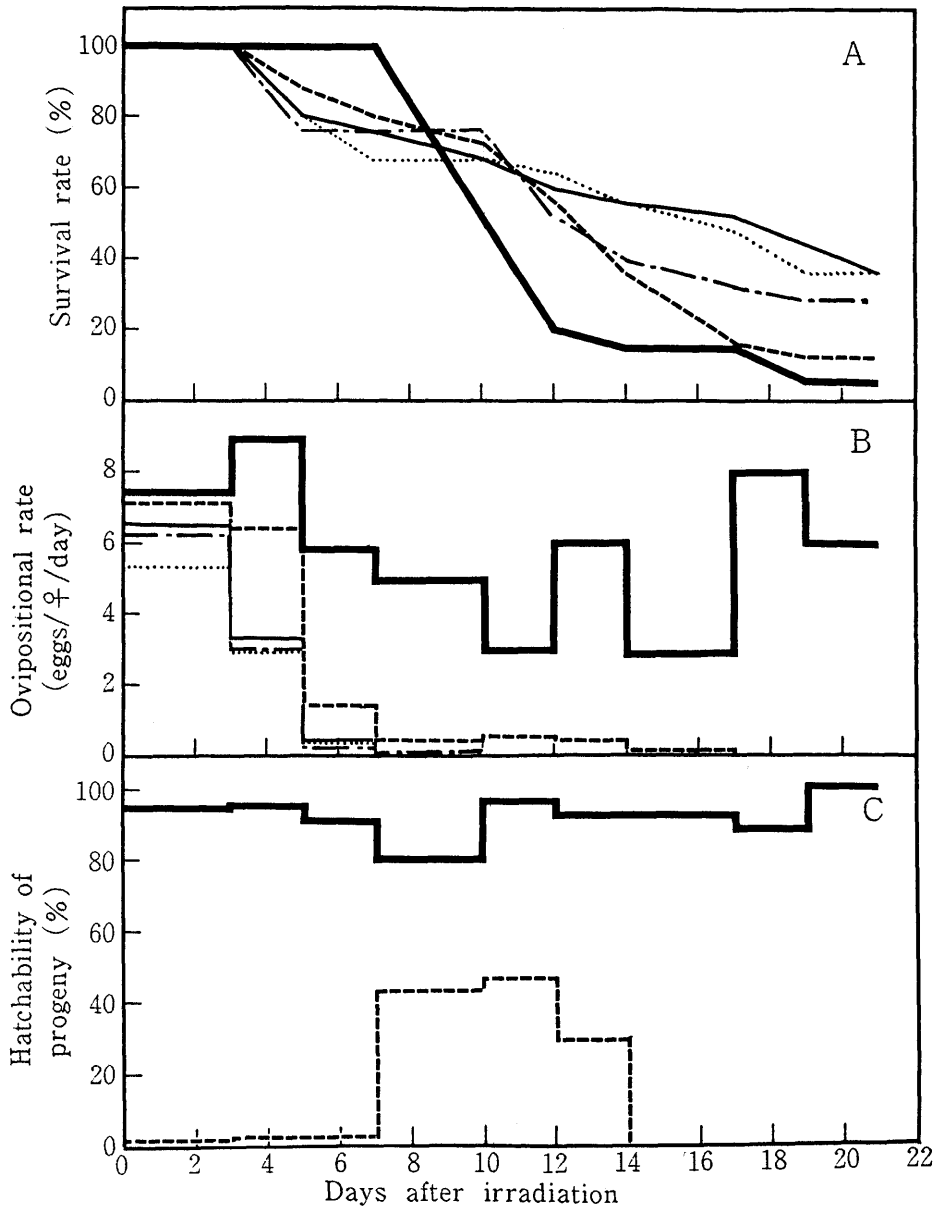

Fig. 3. Survival and ovipositional rate of irradiated adult females, and hatchability of their progeny
——; control, ------; 0.2kGy, — · —; 0.4kGy,
·····; 0.6kGy, ——; 0.8kGy

Discussion

T. urticae eggs increased its tolerance against electron beam irradiation with increased age. A large shift of the tolerance was observed around 3-day-old eggs (Table 1). A similar tendancy was reported for gamma irradiation against *T. urticae* (GOODWIN and WELLHAM, 1990) and *T. arabicus* (ELBADRY and ELAAL, 1972). However, responses of 5-day-old eggs reported by GOODWIN and WELLHAM (1990) were equivalent to that of 4-day-old eggs in our study. For example, the corrected mortality (ABBOTT, 1925) of the former was 58.8% and the latter was 53.6% at 0.6kGy. Since they held newly-laid eggs at 18℃ for 5 days and at 25℃ after irradiation, delayed development of eggs at lower temperatures seems to cause different responses, although they did not mention the status of embryonic development for tested eggs.

In the embryological development of Tetranychidae, extremities completely develop by the time that the eye-spots become visible (DITTRICH, 1965), In the present study, non-irradiated eggs showed red eye-spots 3 days after oviposition. Development of irradiated 1-day-old eggs was stopped before eye-spots were formed, while that of irradiated 2-day-old eggs, which did not hatch out, was delayed and eye-spots appeared 5～7 days after oviposition. Malformed 4th legs were observed on mites from irradiated 3- and 4-day-old eggs. Therefore, the change of tolerance in aged eggs was synchronized with the development of extremities and eye-spots.

Only females emerged from irradiated 5-day-old eggs (Table 2). Eggs fated to be male were obviously susceptible to irradiation. The emergence rate of immature stages from irradiated eggs declined showing two components which consisted of a steep slope in early stages and subsequent gentle slope (Fig. 2). The former slope seems to represent higher susceptibility of male mites. ELBADRY and ELAAL (1972) reported that unfertilized eggs of *T. arabicus* were more sensitive to gamma irradiation than fertilized eggs. In Tetranychidae, fertilized (2n) and unfertilized eggs (n) are fated to be female and male mite, respectively (GUTIERREZ, *et al*, 1970; HELLE and BOLLAND 1967; HELLE, *et al*, 1970; SCHRADER 1923). Therefore, the difference of chromosome number causes the difference in the tolerance to irradiation.

In mated mature females irradiated at 0.2kGy, their fecundity was controlled at low levels (0.6～2.1%) during the first 7 days, but in the next 7 days it temporarily recovered (28.5～46.2%) (Fig. 3-C). Therefore, eggs changed their tolerance before and after oviposition (Table 1, Fig. 3-C). Electron beam irradiation of *Acarus siro* L. showed that spermatogonia and spermatozoa were more tolerant than spermatocytes and spermatids, and suggested that primary gonial cells repopulated testes after irradiation at 0.3kGy and less (SZLENDAK, *et al*, 1992). Variable tolerance of gonial cells in spermatogenesis may be applied to that in oogenesis. In terms of radiation tolerance, egg cells, oogonia and oocytes are presumed to be corresponding to spermatozoa, spermatogonia and spermatocytes, respectively.

Data for dose required to completely sterilize *T. urticae* by gamma irradiation were reported to be 0.32kGy (HENNEBERRY, 1964), 0.25 ～ 0.35kGy (WIT and VRIE, 1985) and 0.3kGy (GOODWIN and WELLHAM, 1990). Present results showed that electron beam irradiation was similar to gamma irradiation in sterile effect. Further studies on larval,

nymphal and diapausing female tolerance, which is unknown, are needed. However, since many species of cut flowers were tolerant to gamma irradiation up to 0.5kGy throughout the year (WIT and VRIE, 1985), ionizing irradiation seems an effective treatment in plant quarantine to control *T. urticae*.

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和 文 摘 要

ナミハダニ *Tetranychus urticae* KOCH (Acari : Tetranychidae) の電子線照射 —卵および雌成虫に与える影響—

土肥野 利幸・田辺 和男
横浜植物防疫所調査研究部調査課

ナミハダニ *Tetranychus urticae* の1～5日齢卵および雌成虫に2.5MeV電子線を照射して，その影響を調べた。

(1) 若齢卵（1～2日齢卵）は老熟卵（3～5日齢卵）よりも感受性が高く，0.2kGyで孵化が阻止された。

(2) 5日齢卵は耐性が最も高く，0.4kGyおよび0.6kGyでは成虫にまで生育したが，これらはすべて不妊化された雌で，0.8kGyでは成虫脱皮が阻止された。

(3) 交尾後の雌成虫を照射したとき，0.2kGyでは一時的な妊性の回復が見られたが，0.4kGyで不妊化された。