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REVIEW ARTICLE

ELECTRON BEAM DISINFESTATION OF CUT FLOWERS AND THEIR RADIATION TOLERANCE

TORU HAYASHI*[1], OLIVIA K. KIKUCHI[2] and TOSHIYUKI DOHINO[3]

[1]National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Kannondai, Tsukuba, Ibaraki, 305, Japan

[2]Energy and Nuclear Research Institute, Travessa R, 400, CEP 05508-970, Sao Paulo, SP, Brazil and

[3]Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Shin-yamashita, Naka-ku, Yokohama, Kanagawa, 231, Japan

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Abstract—Effects of electron beams on spider mite and flour beetle were slightly smaller than those of gamma-rays at equal doses. Electron beams at 400 Gy killed or sterilized all the pests for cut flowers tested; spider mite, mealybug, leaf miner, thrips and cutworm. Carnation, alstromeria, gladiolus, tulip, statice, stock, dendrobium, prairie gentian, oncidium, campanula, gloriosa, fern, gypsophila, freesia, lobelia, triteleia and gerbera were tolerant to electron beams at 400–600 Gy, while chrysanthemum, rose, lily, calla, antherium, sweet pea and iris were intolerant. Radiation-induced deterioration of chrysanthemum could be prevented by post-irradiation treatment with commercial preservative solutions or sugar solutions.

1. INTRODUCTION

Imports of cut flowers to Japan have been rapidly increasing and more than 800 million stalks of cut flowers are imported to Japan. Around 100 million stalks of them do not pass quarantine inspection and are subjected to quarantine treatments, generally fumigation with methyl bromide or cyanide. The fumigation can not treat a large quantity of flowers simultaneously and takes several hours to complete the treatment. Flower traders are interested in the methods that enable to disinfest cut flowers in a short period. Furthermore, fumigation with methyl bromide is not desirable from the viewpoints of both health and environment. The usage of methyl bromide is going to be phased out globally, because of its ozone depleting effect (Anon, 1995, 1996; UNEP, 1995). Thus, the development of disinfestation methods alternative to methyl bromide fumigation is highly required.

One of the methods that can treat a large number of flowers simultaneously as an alternative to methyl bromide fumigation is radiation treatment (UNEP, 1995). Radiation technology as a quarantine treatment will be used to inactivate not only insects but also mites, spider mites, thrips, nematodes, snails, and slugs contaminating grains, fruits, vegetables, cut flowers, fresh herbs, timbers, seedlings and seeds, if it is implemented. Data on the effects of radiation on fruit flies and stored-product insects and on grains, fruits and vegetables have been accumulated (Anon, 1995). However, data on other pests and host commodities are not enough to evaluate radiation technology as a quarantine treatment (Anon, 1996), although a few studies have been conducted on radiation tolerance of cut flowers (Haasbroek *et al*., 1973; Joyce, 1988; Seaton and Joyce, 1992; Wit and Van de Vrie, 1985). FAO/IAEA has conducted an international project to determine the minimum doses necessary to inactivate pests other than fruit flies and stored-product insects and the maximum doses cut flowers and fresh herbs tolerate since 1992 (Anon, 1996).

Quarantine treatments are carried out at airports as well as at ports. Electron irradiators at airports are preferable to gamma-ray irradiators with ^{60}Co, when accidents such as plane crash are taken into consideration. The Japanese Government has conducted a research project on the disinfestation of cut flowers with electron beams since 1991. The insecticidal effects of electron beams were compared with those of gamma-rays, and the doses of electrons necessary to inactivate pests of cut flowers and the doses cut flowers tolerate were determined. The methods to prevent radiation-induced deterioration of cut flowers were examined as well.

*To whom all correspondence should be addressed.

Table 1. Sterility of flour beetle, *Tribolium freemani*, adult females from pupae irradiated with gamma-rays or elctron beams

	Egg laid (%)		Egg hatched (%)	
Dose (Gy)	Gamma-ray	Electron beam	Gamma-ray	Electron beam
100	0	0	—	—
50	0	13.3	—	0
25	40.0	88.2	16.1	44.6
10	93.3	93.3	55.8	73.9
0	85.0		82.7	

2. COMPARATIVE EFFECTS OF GAMMA-RAYS AND ELECTRON BEAMS ON PESTS

The effects of gamma-rays and electron beams on flour beetle, *Tribolium freemani*, and two spotted spider mite, *Tetranychus urticae*, were investigated and compared by using a Gammacell 220 (4.7 kGy/h, 2.1×10^2 TBq of ^{60}Co, Nordion International Inc., Canada) and a Van de Graaff electron accelerator (1.2×10^3 kGy/h, 2.5 MV, Nissin High Voltage Engineering Co., Ltd, Japan).

A smaller number of adults from gamma-irradiated pupae of flour beetle laid eggs, as compared with the adults from electron-irradiated pupae (Table 1). The hatchability of eggs laid by the adults from gamma-irradiated pupae was slightly lower than that from electron-irradiated pupae (Table 1). Younger eggs of two spotted spider mite were more sensitive to both gamma-rays and electron beams (Table 2). The effect of gamma-rays on the hatchability of the eggs was almost the same as that of electron beams (Table 2) (Dohino *et al*., 1994). These results (Table 1 and Table 2) indicate that the insecticidal effects of gamma-rays are slightly larger than those of electron beams, which agreed with the results reported on other insects (Adem *et al*., 1978; Bull and Cornwell, 1966; Hayashi, 1991; Proctor *et al*., 1954).

3. EFFECTS OF ELECTRON BEAMS ON PESTS

All pests at each stage were irradiated at ambient temperature with the van de Graaf electron accelerator.

Table 2. Hatchability of two spotted spider mite, *Tetranychus urticae*, eggs irradiated with gamma-rays or electron beams (%). Uncertainties at 1 s.d.

Age of egg	Dose (Gy)	Electron beam	Gamma-ray
3 day-old	50	90.4 ± 6.3	88.3 ± 11.2
	100	89.4 ± 6.8	88.6 ± 6.7
	150	84.1 ± 11.8	82.8 ± 7.8
	200	69.6 ± 12.8	65.4 ± 7.8
4 day-old	200	97.7 ± 3.8	95.7 ± 4.8
	400	96.0 ± 4.3	84.7 ± 7.8
	600	77.5 ± 9.4	76.8 ± 13.3
	800	52.6 ± 16.7	50.7 ± 13.9
	1000	29.7 ± 20.2	23.4 ± 22.2

From Dohino, T., Tanabe, K. and Hayashi, T. (1994) *Res. Bull. Pl. Prot. Jap.* **30,** 69.

3.1. *Spider mite*

Two spotted spider mite, *Tetranychus urticae*, was reared on kidney bean leaves at 22°C and 70% r.h. under an illumination condition of 10 μmol·m^{-2}·s^{-1} PPF for 16 h and darkness for 8 h. The durations of egg, and larval and nymphal stages were 4–7 days and 7–9 days, respectively. Adult females were obtained about 9 days after hatching.

The tolerance of eggs increased with age. The most tolerant eggs, 5-day-old eggs, irradiated at 400 and 600 Gy developed into adults, but the adult females were sterilized completely. Adult females irradiated at 400 Gy or higher did not produce viable eggs, although females irradiated at 200 Gy recovered fecundity. (Dohino and Tanabe, 1993).

3.2. *Leafminer*

Twenty female flies of leafminer, *Liriomyza trifolii*, were released in a cage that contained three kidney bean seedlings. The flies were allowed to lay eggs on the leaves freely for 6 hours. After exposure, the leaves were cut at the petioles and moved to petri dishes with agar. The cut end of the leaf was put into agar to prevent the leaf from drying. The leaves were kept at 25°C under an illumination condition of 10 μmol·m^{-2}·s^{-1} PPF to develop larvae, pupae and adults. Eggs, larvae and pupae on leaves were irradiated with electron beams.

Irradiation of eggs, larvae and pupae at 100 Gy or higher inhibited adult emergence. (Kumagai and Dohino, 1995).

3.3. *Mealybug*

Comstock mealybug, *Pseudococcus comstocki*, was reared on pumpkin at 23°C and 70% r.h. in darkness. The developmental duration of egg was 12 days. Adult males emerged in 30 days and adult females laid eggs in 50 days after hatching.

Younger eggs (0–5 day-old and 6–10 day-old) were more sensitive to electron beams than older eggs (11 or 12 day-old). Larvae irradiated at 200 Gy did not grow into maturity. Adult females irradiated at 200–600 Gy oviposited, but the eggs did not hatch except at 200 Gy. Female descendents from eggs irradiated at 200 Gy maintained the fecundity (Dohino and Masaki, 1995).

3.4. *Cutworm*

Seven or eight adult pairs of tobacco cutworm, *Spodoptera litura*, were allowed to oviposit on the

inside a tracing paper bag at 25°C and 60–80% r.h., under an illumination condition of 10 $mmol \cdot m^{-2} \cdot s^{-1}$ PPF for 16 h and darkness for 8 h. The eggs were put in a petri dish and held under the above conditions for preparing 1, 2, 3 and 4 day-old eggs and larvae. Larvae were given an artificial diet (Insecta LF, Nihon Nosan Kogyo K.K., Japan) in a plastic container (21 × 29 × 10 cm). Third instar larvae (3rd instars were obtained 6–7 days after hatching) and fifth instar larvae (5th 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.

Older eggs were more resistant to electron beams. Four day-old eggs hatched after irradiation at 400 Gy, although the other eggs did not. Larvae from eggs irradiated at 400 Gy did not develop into 2nd instars. Larvae irradiated at 100 Gy or higher did not develop into adults (Dohino *et al*., 1996a).

3.5. *Thrips*

Thrips palmi and *Thrips tabaci* were reared with cucumber leaves in a cage which was covered with Bemberg nets (Asahi Chemical Industry Co. Ltd, Japan) at 25°C and 60–80% r.h., under an illumination condition of 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF for 16 h and darkness for 8 h. Under these conditios, the durations of egg, larval stage and pupal stage were 4–5 days, 4–6 days and 4–5 days, respectively. Ten adult females were allowed to oviposit into a cucumber leaf that was put on a filter paper in a polyethylene bag. Collection of eggs of *T. palmi* and *T. tabaci* was performed for 3 days and for 1 day, respectively. The eggs laid in the cucumber leaves were held under the rearing conditions until irradiation. Second instar larvae and adults for irradiation were collected from the colonies in a rearing cage and placed on a cucumber leaf in a polyethylene bag.

The resistance of eggs to radiation increased with age. Eggs irradiated at 100 Gy or higher did not grow into pupae, irrespective of the age. Adult emergence from larvae of *T. tabaci* was inhibited at 200 Gy, but that from larvae of *T. palmi* was not inhibited even at 400 Gy. However, the adults emerged from larvae irradiated at 100 Gy or higher were sterilized. Irradiation of adults of the two species of thrips at 400 Gy resulted in sterilization (Dohino *et al*., 1996b).

Irradiation with electron beams at 400 Gy inhibited the hatching, larval growth, pupation, adult-emergence and/or oviposition and sterilized the adults, irrespective of the species and stage of pest, which suggests that 400 Gy is the minimum dose for inactivation of pests for cut flowers with electron beams.

4. EFFECTS OF ELECTRON BEAMS ON CUT FLOWERS

Flowers were irradiated with a Dynamitron (5.0 MV, Radiation Dynamics Inc., U.S.A.). Irradiated flowers were soaked in distilled water and stored at 20°C.

Carnation, alstromeria, gladiolus, tulip, statice, stock, dendrobium, prairie gentian, oncidium, campanula, gloriosa, fern, gypsophila, freesia, lobelia, triteleia and gerbera were tolerant to electron beams at 400 Gy, while chrysanthemum, rose, lily, calla, antherium, sweet pea and iris were intolerant. The detrimental effects of irradiation were delay/inhibition of flowering, withering/browning of flowers and leaves and bending of petioles. (Kikuchi *et al*., 1995; Tanabe and Kato, 1992; Tanabe and Dohino, 1993, 1995)

Table 3. Quality of irradiated chrysanthemums held in various vase solutions for 28 days at 25°C. Uncertainties at 1 s.d.

Vase solution	Onset of flower wilting (days)[z]	Onset of leaf yellowing (days)[y]	Flowers fresh weight (g)[x]	Fraction of yellowed leaves (%)[x]
Water	8.3 ± 2.2	5.9 ± 0.9	3.17 ± 0.51	100.0 ± 0.0
0.02% HQS	8.5 ± 2.5	5.5 ± 1.8	3.56 ± 0.48	100.0 ± 0.0
0.1% Sorbate	7.9 ± 1.8	6.1 ± 1.5	3.33 ± 0.79	100.0 ± 0.0
0.03% DBS	8.0 ± 2.2	6.2 ± 1.1	3.75 ± 0.81	100.0 ± 0.0
0.01% PEL	8.3 ± 2.5	6.3 ± 1.6	3.55 ± 0.76	100.0 ± 0.0
0.024% STS	7.8 ± 2.6	5.9 ± 1.8	3.78 ± 0.79	100.0 ± 0.0
0.01% BA	8.2 ± 2.7	5.5 ± 1.8	3.88 ± 0.68	100.0 ± 0.0
0.001% GA	8.6 ± 2.4	5.9 ± 1.8	3.68 ± 0.88	100.0 ± 0.0
2% Glycerol	7.9 ± 2.5	5.7 ± 1.8	3.21 ± 0.92	100.0 ± 0.0
2% Mannitol	7.8 ± 2.8	5.8 ± 1.6	3.59 ± 0.78	100.0 ± 0.0
2% Sorbitol	8.3 ± 2.9	5.8 ± 1.7	3.03 ± 0.87	100.0 ± 0.0
2% Sucrose	22.0 ± 1.8	19.9 ± 2.6	13.56 ± 0.84	8.0 ± 4.8
2% Glucose	21.5 ± 1.4	19.2 ± 2.1	13.25 ± 0.78	8.8 ± 3.6
2% Fructose	22.6 ± 1.5	19.1 ± 1.9	12.98 ± 0.91	8.0 ± 3.2
2% Maltose	22.6 ± 1.4	18.9 ± 2.3	13.32 ± 0.89	8.8 ± 4.0
Sucrose + HQS	22.0 ± 1.7	18.2 ± 2.2	12.58 ± 1.09	10.0 ± 3.6
Sucrose + sorbate	22.5 ± 1.6	19.6 ± 1.9	13.35 ± 0.85	10.4 ± 4.4
Control	22.3 ± 1.7	19.4 ± 2.0	7.93 ± 0.83	9.6 ± 5.2

Control; unirradiated chrysanthemums held in water.
[z]Days after irradiation when the first wilted flower was observed.
[y]Days after irradiation when the first yellowed leaf was observed.
[x]Determined 21 days after irradiation.
From Hayashi, T. and Todoriki, S. (1996) *HortScience* **31,** 117.

Table 4. Quality of irradiated chrysanthemums treated with sucrose before and during and/or after iradiation and held for 28 days at 25°C. Uncertainties at 1 s.d.

Treatment	Onset of flowers wilting (days)[z]	Onset of leaf yellowing (days)[y]	Flower fresh weight (g)[x]	Fraction of yellowed leaves (%)[x]
Water-water[w]	8.2 ± 2.3	5.7 ± 1.7	3.09 ± 0.57	100.0 ± 0.0
2% Sucrose-water[w]	7.9 ± 2.1	6.2 ± 1.3	3.25 ± 0.59	100.0 ± 0.0
2% Sucrose-2% sucrose[w]	22.5 ± 1.8	18.2 ± 2.3	13.56 ± 1.05	8.8 ± 4.8
Water-2% sucrose[w]	21.6 ± 1.5	19.1 ± 2.1	12.85 ± 0.97	11.6 ± 5.2
Control	22.9 ± 1.8	19.8 ± 1.9	8.25 ± 0.87	11.2 ± 6.0

Control: unirradiated chrysanthemums held in water.
[z]Days after irradiation when the first wilted flower was observed.
[y]Days after irradiation when the first yellowed leaf was observed.
[x]Determined 21 days after irradiation.
[w]Stems were held in the first solution before and during irradiation, and in the second solution after irradiation.
From Hayashi, T. and Todoriki, S. (1996) *HortScience* **31,** 117.

5. PREVENTION OF RADIATION-INDUCED DETERIORATION

Flowers were soaked in various vase solutions for 12 h and irradiated at 750 Gy with the gammacell in the same vase solutions, followed by storage at 20°C in the vase solutions.

Commercial floral preservative solutions and aqueous solutions (2%) of sucrose, glucose, fructose and maltose delayed bloom wilting and foliage yellowing of cut chrysanthemums caused by irradiation at 750 Gy; irradiated chrysanthemum stems placed in such sugar solutions showed almost the same vase-life as unirradiated stems placed in water (Table 3) (Hayashi and Dohino, 1995; Dohino and Hayashi, 1995; Hayashi and Todoriki, 1995, 1996; Kikuchi *et al*., 1995). Solutions of 8-hydroxyquinoline sulfate (HQS), silver thiosulfate (STS), sodium dodecylbenzenesulfonate (DBS), polyoxyethylene lauryl ether (PLE), potassium sorbate, mannitol, sorbitol, glycerol, 6-benzylamino purine (BA) and gibberelline (GA) did not reduce radiation-induced deterioration. The results indicate that only sugars were effective in preventing radiation-induced deterioration and that germicides, polyols, surfactants, silver ion and plant hormones were not effective. Sugar solutions prevented foliage yellowing of rose brought about by irradiation but not flower wilting.

Placing chrysanthemum stems in 2% sucrose before and during irradiation did not influence the vase-life, but placing chrysanthemum stems in 2% sucrose following irradiation prolonged the vase-life (Table 4) (Hayashi and Dohino, 1995; Hayashi and Todoriki, 1996). These results indicate that sugars influence post-irradiation metabolism responsible for radiation-induced deterioration of chrysanthemum cut flowers.

6. CONCLUSION

Effects of electron beams on insects were slightly smaller than those of gamma-rays, so dose necessary to disinfest cut flowers with electron beams will be slightly higher than that with gamma-rays. All the pests tested in these studies could be inactivated by electron beams at 400 Gy. Some cut flowers were tolerant to electron beams at 400 Gy and others were intolerant; electron-irradiation is practical for the flowers that are not damaged at 400 Gy. Radiation-induced deterioration of chrysanthemums can be prevented by sugar solutions. However, no method has been developed to prevent detrimental effects of radiation on other flowers.

Although the efficacy of electron-irradiation of some cut flowers as a quarantine treatment has been confirmed, there are still a few problems to be solved. The dose necessary to inactivate pests is slightly lower than that cut flowers tolerate. The difference between the two doses is not large enough to allow treating flowers with electron beams with low penetration, because the difference between the two doses would be smaller than the difference between maximum and minimum doses of electrons inside a carton of flowers. X-rays are preferable to electron beams for treating cut flowers for quarantine purposes. However, technology to irradiate products continuously for a long period with X-rays has not been established.

Most of the irradiated insects do not die immediately after irradiation and the surviving insects will get into importing countries with irradiated cut flowers. Pests such as aphids and thrips are sometimes infected with plant virus, so insects that survive after irradiation may bring virus into importing countries. The possibility for irradiated insect to be a vector of virus has not been investigated.

When cut flowers are irradiated in exporting countries, the irradiation treatment should be confirmed by the authorities of importing countries. During the transportation of irradiated products from exporting countries to importing ones, the products may be re-contaminated with pests. Detection methods of irradiated pests will be helpful to confirm that all the living pests contaminating fresh agricultural products have been irradiated. However, few such methods have been reported (Anon, 1996).

Efforts should be made to solve these new problems in addition to the confirmation of the efficacy of irradiation as a quarantine treatment in order to establish quarantine techniques with radiation.

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