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Effect of Gamma-irradiation and Heat on Root-knot Nematode, *Meloidogyne javanica*[1]

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Abstract: Effects of gamma-irradiation on the root-knot nematode *Meloidogyne javanica* were investigated. A dose of 7.5 kGy killed all second-stage juveniles (J2) within 1 day after treatment. Egg hatch was completely inhibited at 6.25 kGy. A bioassay on tomato measuring galling and egg production was used to determine the infectivity of irradiated J2 and J2 hatched from irradiated eggs. The J2 and eggs irradiated with a dose of 4.25 kGy did not induce galls or reproduce on tomato plants. When nematodes were exposed to combined irradiation and heat treatment, no synergistic effect on J2 or eggs was measured. Heat treatment at 49 °C for 10 minutes or 20 minutes without irradiation immobilized J2 and prevented egg development. Irradiation rates needed to kill or incapacitate *M. javanica* were high and may be impractical as a quarantine measure.

Key words: Cobalt-60, control, dose, gamma-irradiation, heat treatment, kGy, *Meloidogyne javanica,* quarantine, root-knot nematode.

Nematode species vary greatly in their sensitivity to irradiation (Evans, 1970; Myers, 1960; Townshend, 1967; Yeargers, 1981). Most of those examined have survived doses up to 3.2 kGy without serious effects on their population densities. Ishibashi and Taguchi (1965) found that irradiation of juveniles of *Meloidogyne incognita* at 1.5 kGy did not cause sex reversal of third-stage juveniles. In another study, however, Ishibashi (1965) reported that when undifferentiated juveniles of *M. incognita* were irradiated at doses of 0, 0.1, or 0.2 kGy 3 days after penetrating plant roots, 1.5%, 18.3%, and 27.2%, respectively, developed into single-gonad males. Irradiation of *M. incognita* with cotyloid to V-shaped gonads with 0.1 and 0.2 kGy increased the numbers of sex-reversed males by 24.9% and 33.5%, respectively, compared to those not irradiated. Furthermore, irradiation retarded the growth of nematodes in proportion to the dose. Irradiation of second-stage juveniles (J2) soon after root penetration retarded their growth more than irradiation of later-stage juveniles.

All *Pratylenchus thornei* were eliminated from the soil at 7.5 kGy or higher, but approximately 1% of the nematodes survived at a dose of 5 kGy (Thompson, 1990). Lethal doses of gamma-irradiation (Cesium-137) for *Bursaphelenchus xylophilus* were between 6 to 8 kGy (Eicholz et al., 1991). Unfortunately, these dosages were too high for irradiation to be practical for disinfesting wood chips commercially.

Irradiation doses required to kill or sterilize root-knot nematodes (*Meloidogyne* spp.) in all stages of development are unknown. In preliminary studies in Thailand on the effect of irradiation on *M. incognita,* J2 and egg masses were exposed to doses of 1.2 and 1.5 kGy, respectively. After 14 days, J2 were not killed and eggs were able to hatch (Chinnasri, unpubl.). Bioassays revealed that irradiated J2, as well as J2 from irradiated eggs, induced root galling and reproduced on tomato (Chinnasri, unpubl.).

Irradiation may be an alternative to fumigation for disinfestation of plant material. If it is cost effective, irradiation would be relatively safe to the environment and man. The objectives of this research were to determine (i) the effect of irradiation on J2 and eggs of *M. javanica,* and (ii) the effect of a combination of irradiation and heat treatment on nematode reproduction.

Materials and Methods

The *M. javanica* isolate was propagated on tomato (*Lycopersicon esculentum* cv. Rutgers)

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in a greenhouse (30 ± 5 °C). Egg masses were hand picked from 45-day-old cultures. J2 were collected by shaking roots in 0.5% sodium hypochlorite solution (Hussey and Barker, 1973) and incubating eggs on hatching screens at 25 °C for 24 hours. Two experiments were conducted in the laboratory and greenhouse.

Irradiation of Meloidogyne javanica: Several doses of irradiation were tested to determine effects on mortality, hatch, and infectivity of *M. javanica.* For the mortality test, approximately 100 1-day-old J2 were placed in 17- × 60-mm vials containing sterile distilled water and exposed to gamma-irradiation (Cobalt-60 source of 4,943 curies and a dose rate of 0.009930 kGy/minute) for 0 to 14.5 hours to give doses of 0, 2, 4, 6, 7, 7.5, and 8 kGy. The treatments were replicated three times. Second-stage juveniles were assumed dead when they were observed to lie motionless in a straightened position, be swollen, or have turned dark and lost internal integrity. Juveniles were observed daily, and those presumed dead were counted and recorded.

For egg hatch tests, egg masses were placed individually into 17- × 60-mm vials containing sterile distilled water and irradiated by gamma-radiation (Cobalt-60 source of 4,943 curies and a dose rate of 0.009930 kGy/minute) at doses of 0, 2, 3, 4, 5, 6, 6.25, and 6.5 kGy. The irradiated egg masses were transferred from the vials into 5-cm-diam. petri dishes filled with sterile distilled water and incubated at room temperature (25 °C). Dishes were examined daily, and J2 were counted. At day 15, the gelatinous matrix covering the eggs was dissolved with 0.5% sodium hypochlorite solution (Hussey and Barker, 1973), and the unhatched eggs were counted. The total number of eggs per egg mass was determined by adding the J2 count to the unhatched egg count. This total was used to calculate the percentage hatch. There were three replicates per irradiation dose.

For the infectivity test, aliquots of 5,000 1-day-old J2 or of 20 egg masses were placed in 17- × 60-mm vials containing sterile distilled water and irradiated at doses of 0, 1, 2, 3, 3.25, 3.5, 3.75, 4, 4.25, and 4.5 kGy using Cobalt-60, a source of 4,832 curies providing a dose rate of 0.009708 kGy/minute. The irradiated juveniles were then inoculated onto 5-cm-tall Rutgers tomato seedlings in 10-cm-diam. clay pots. The irradiated egg masses were observed under a microscope to ensure that there was no contamination by any newly hatched juveniles. The egg masses were then transferred into another vial containing sterile distilled water and inoculated onto a 5-cm-tall Rutgers tomato seedling in a 10-cm-diam. clay pot. Inoculated tomato was arranged in a randomized complete block (RCB) with five replicates per treatment, two plants per replicate, and maintained in the greenhouse. Forty-five days after inoculation, the tomato roots were given a 0 to 5 gall rating where 0 = no galling, 1 = trace infections with a few small galls, 2 = less than 25%, 3 = 26–50%, 4 = 51–75%, and 5 = greater than 75% of roots galled (Kinloch, 1990). Eggs were extracted from tomato roots using a 0.5% sodium hypochlorite solution (Hussey and Barker, 1973). The number of eggs per gram of wet root weight was calculated.

Irradiation and heat treatment of M. javanica: Two 4 × 4 factorial arranged experiments were conducted—one on J2 and the second on egg masses. Approximately 5,000 1-day-old J2 or 20 egg masses were placed in 17-×-60-mm vials containing sterile distilled water. The vials were immersed in a water bath and exposed to one of three heat treatments: 43 °C for 10 minutes, 49 °C for 10 minutes, or 49 °C for 20 minutes. A room temperature control also was included. Immediately after the heat treatment, the J2 or egg masses were exposed to gamma-radiation doses of 0, 0.005, 0.01, or 0.015 kGy (Cobalt-60 source of 4,832 curies and a dose rate of 0.009708 kGy/minute). The heat-treated and irradiated juveniles and egg masses were then inoculated onto 5-cm-tall Rutgers tomato seedlings grown in 10-cm-diam. clay pots and maintained in a greenhouse. The treatments were replicated three times, two plants per replicate. All tomato plants were harvested 45 days after inoculation. Roots were rated for galling (Kin-

loch, 1990) and eggs were extracted from roots with sodium hypochlorite (Hussey and Barker, 1973). Eggs were counted and final nematode population densities calculated.

Results

Irradiation of Meloidogyne javanica: Second-stage juveniles appeared unaffected by irradiation at 4 kGy and below (Fig. 1). Based upon loss of spontaneous motility, increasing J2 mortality occurred as irradiation doses increased above 4 kGy (Fig. 1). Mortality of the J2 irradiated at a dose of 2 or 4 kGy was low and did not differ from mortality of those J2 not exposed to gamma-irradiation ($P = 0.01$). Irradiation with 6 kGy resulted in greater nematode mortality than in the control 5 days after exposure ($P = 0.01$) and then increased to 80% by 15 days after treatment. With a dose of 7 kGy, some mortality was observed by the second day and increased rapidly; all nematodes were dead 5 days after exposure (Fig. 1). Mortality was 100% the day following exposures to 7.5 and 8 kGy (Fig. 1).

In the egg irradiation test, hatching decreased with increasing irradiation at all doses tested (Fig. 2). The number of J2 hatching from egg masses irradiated at doses ranging from 4 to 6 kGy was low throughout the 15 days of the experiment. Doses of 6.25 and 6.5 kGy completely inhibited egg hatch (Fig. 2).

The infectivity assay proved to be a more sensitive indicator of the effect of irradiation on *M. javanica* juveniles and eggs than the observations on mortality and hatch (Table 1). A dose as low as 2 kGy reduced root galling and the number of eggs per gram of root (Table 1). Eggs were less sensitive than J2 to irradiation. Reduction in galling and numbers of eggs per gram of root did not occur below doses of 3.25 kGy. Exposure of J2 and egg masses to 4.25 kGy or greater resulted in no galling and no measurable egg production.

Irradiation and heat treatment of M. javanica: The low irradiation doses evaluated did not affect galling or numbers of eggs per gram of root on tomato ($P > 0.05$). Heat treatments, however, reduced both galling and nematode reproduction (Table 2). There was no interaction between irradiation and

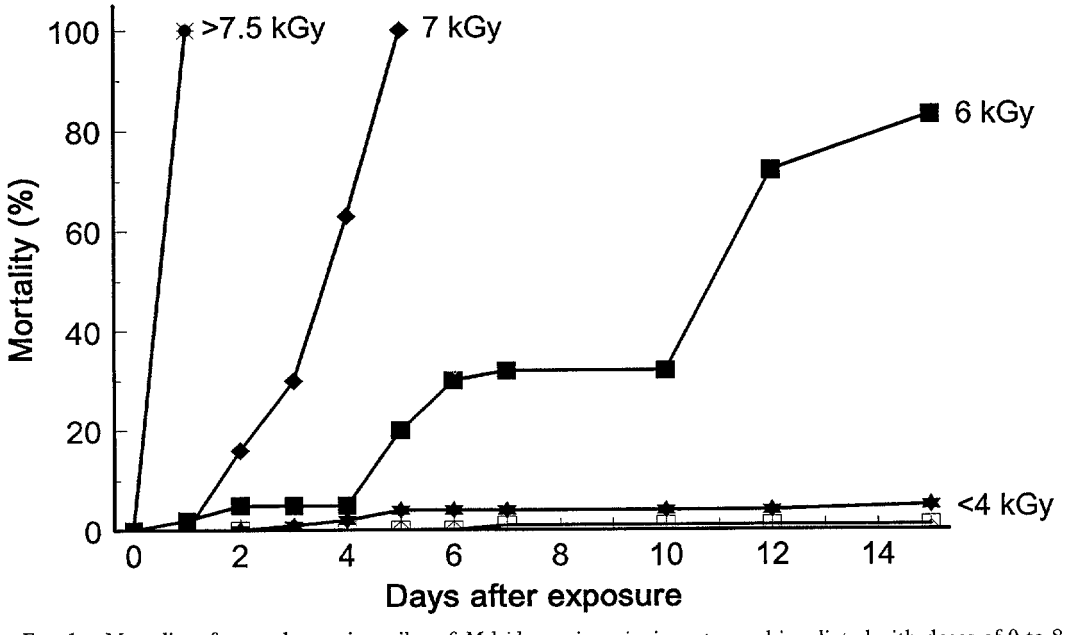

Fig. 1. Mortality of second-stage juveniles of *Meloidogyne javanica* in water and irradiated with doses of 0 to 8 kGy (Cobalt-60 source of 4,943 curies and a dose rate of 0.009930 kGy/minute). Data are means of three replications.

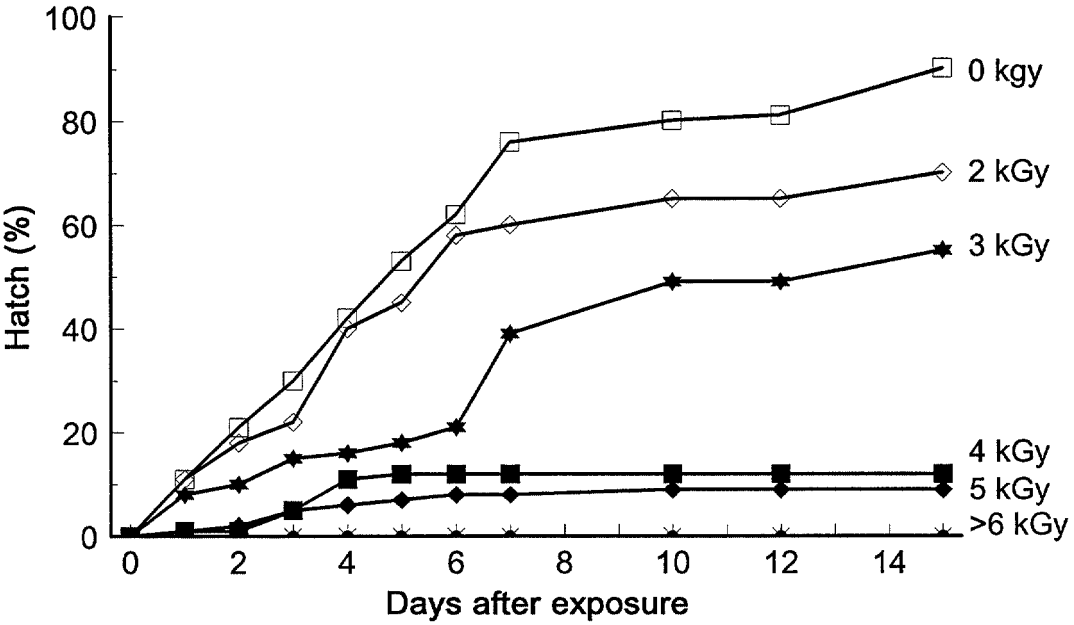

FIG. 2. Effect of irradiation with Cobalt-60 at increasing doses (4,943 curies and a dose rate of 0.009930 kGy/minute) on hatch of *Meloidogyne javanica* over 15 days after exposure. Means are from three replications.

heat treatment ($P > 0.05$). Reproduction was completely inhibited by the 49 °C heat treatment after 10 or 20 minutes (Table 2). J2 were adversely affected by the 43 °C treatment as measured by the subsequent reduction in root-gall index (Table 2). In contrast, heat treatment at 43 °C for 10 minutes was insufficient to inactivate eggs.

TABLE 1. Root galling and reproduction of *Meloidogyne javanica* second-stage juveniles (5,000) or eggs (20 egg masses) exposed to various irradiation doses (Cobalt-60 source of 4,943 curies and a dose rate of 0.009930 kGy/minute) 45 days after inoculation onto tomato.

	Inoculum source			
	J2		Eggs	
Irradiation dose (kGy)	Gall index	Eggs/g root	Gall index	Eggs/g root
0	5.0 a	202 a	4.4 a	201 a
1	5.0 a	185 a	4.8 a	135 ab
2	4.0 b	143 b	4.5 a	146 ab
3	3.4 c	83 c	4.6 a	94 b
3.25	2.7 d	20 d	2.3 b	52 c
3.50	2.6 d	13 e	2.3 b	15 d
3.75	1.8 e	14 e	1.4 c	10 d
4	1.0 f	9 f	0.5 d	4 e
4.25	0 g	0 f	0 d	0 f
4.50	0 g	0 f	0 d	0 f

Means are of five replicates, two plants per treatment. Means followed by the same letter in a column are not different according to Duncan's multiple-range test ($P \leq 0.05$).

Galling was rated on a 0 to 5 scale (0 = no galling, 1 = trace infections with a few small galls, 2 = less than 25%, 3 = 26–50%, 4 = 51–75%, and 5 = greater than 75%).

DISCUSSION

Irradiation doses required to kill J2 or inhibit hatch of *M. javanica* were comparatively high and beyond the tolerance limit of most plants. A principle of irradiation on living organisms is that the simpler the organism is, the more resistant it is to irradiation (Moy, 1994). Sublethal doses of irradiation affected *M. javanica* viability. To enhance the efficacy of sublethal exposures, the nematodes were stressed with heat before they were irradiated. However, the heat stress was sufficient in itself to kill the nematodes and failed to increase sensitivity to the irradiation.

One of the most important considerations for the control of nematodes with irradiation is the effect of irradiation on plants (O'Bannon, 1992). The treatment must not adversely affect plant growth. Applicability of irradiation on crops such as ginger rhizomes or taro cormels for disinfestation of

TABLE 2. Effect of heat treatment on the capability of second-stage juveniles (J2) and eggs of *Meloidogyne javanica* to reproduce on tomato as measured by root-gall index and final population of eggs (Pf) 45 days after inoculation. Nematodes (5,000) and egg masses (20) were exposed to the heat treatment in vials containing sterile distilled water, inoculated, and assayed 45 days later.

	J2		Eggs	
Heat treatment	Root-gall index	Pf	Root-gall index	Pf
Control	5.0 a	24,815 a	5.0 a	27,835 b
43 °C for 10 minutes	3.9 b	18,920 a	4.9 a	33,670 a
49 °C for 10 minutes	0 c	0 b	0 b	0 c
49 °C for 20 minutes	0 c	0 b	0 b	0 c

Numbers are means of 6 replications. Numbers in a column followed by the same letter are not different according to Fisher's protected LSD. ($P \leq 0.01$).

Galling was rated on a 0 to 5 scale (0 = no galling, 1 = trace infections with a few small galls, 2 = less than 25%, 3 = 26%–50%, 4 = 51–75%, and 5 = greater than 75%).

Meloidogyne is dependent upon the quality of the rhizomes and cormels after treatment. Ginger rhizomes and taro cormels are inhibited from sprouting by irradiation doses of 0.025 and less than 0.025 kGy, respectively (Yusof, 1990; Chinnasri, unpubl.). Disinfestation, fortunately, does not require elimination of the nematode from the plant material. Prevention of nematode reproduction is sufficient (Ouye and Gilmore, 1985).

Currently, plant-parasitic nematodes remain target pathogens of regulatory programs in many countries. They are quarantined and subjected to importation bans on infected plant material. The development of control methods that assure importation of nematode-free planting materials and soil is urgently needed. Additional research into the biological effects of irradiation on nematodes will assist in the selection of effective combination treatments of irradiation and chemical or physical methods (Roberts and Matthews, 1996).

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