THE EFFECTS OF GAMMA IRRADIATION ON THE MOTILITY, INFECTIVITY, REPRODUCTION AND MORPHOLOGY OF DITYLENCHUS DIPSACI 1)

BY

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The development of fourth-stage larvae of Ditylenchus dipsaci after exposure to gamma irradiation at dosages from 16 to 400 kilorads was studied monoxenically. Nematode motility 6 hours after irradiation was significantly higher in controls than in treatments receiving doses of 48, 96 and 192 kilorads; by the fourth day this difference in motility disappeared. Severity of shoot crown swelling in alfalfa seedlings decreased with an increase in irradiation dose to the inoculum. Population increase ceased in cultures inoculated with nematodes irradiated with doses of 48 kilorads and greater. Nematode population increases were greater when the inoculum was irradiated in a nitrogen rather than in an oxygen atmosphere. Several morphological abnormalities were present only in the progenies of nematodes irradiated at dosages of 32, 48, 64, 80 and 96 kilorads.

Plant- and animal-parasitic nematodes which have been investigated are known to be more radio-resistant than the arthropods and other higher animals (Gomberg & Gould, 1953; Myers & Dropkin, 1959). The use of ionizing radiations in nematode control programs is as yet of limited value. The effect of low dosages of gamma irradiation is more conspicuous in the progeny of irradiated nematodes because of the injurious effects to actively dividing germ cells (Myers, 1960; Wood & Goodey, 1957). Irradiation of Meloidogyne incognita second-stage larvae before sexual differentiation causes retardation of gonad growth in the adults of the same generation (Ishibashi, 1965). Sterilization, reduction in reproductive potential, complete or partial retardation of larval emergence, sex reversal and various morphological abnormalities are some of the consequences of gamma irradiation of nematodes (Fassuliotis, 1958; Ishibashi, 1965; Myers, 1960; Wood & Goodey, 1959; Weischer, 1957).

The effects of gamma irradiation on the host-parasite relationships of Ditylenchus dipsaci and alfalfa were evaluated under monoxenic conditions by comparison of the (1) host response to irradiated and non-irradiated nematodes; (2) population levels and structures of the parasites irradiated with various dosages; and (3) effect of irradiation on the morphology of the treated animals and their subsequent generations.

Hypoxia often increases the radio-resistance approximately by a factor of two

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(Casarett, 1968), therefore, the effects of irradiation on *D. dipsaci* in oxygen and nitrogen atmospheres were compared.

**MATERIALS AND METHODS**

Nematode inocula used in this study were obtained from monoxenic cultures of *D. dipsaci* (alfalfa race) maintained on El Dorado alfalfa (*Medicago sativa*) seedlings. The onion race of *D. dipsaci* was obtained from heavily infected garlic scales. The nematodes, predominantly fourth-stage larvae and a few adults, were axenized with 130 ppm aretan (16 hours), 2,000 ppm streptomycin sulfate and 2,500 units/ml penicillin (8 hours) using the method of Lownsbery & Lownsbery (1956).

Samples prepared of approximately 5,000 axenic nematodes each in 5 ml glass vials were irradiated in a pool-type 32,500 c Cobalt-60 source at the University of California, Davis. The dose rate was 250 kilorads/hour. Dosimetry was determined by Dr. R. J. Romani and his staff in the Department of Pomology. The dosages employed were 16, 32, 48, 64, 80, 96, 128, 160, 192, 224, 256, 288, 320 and 400 kilorads.

In these experiments the nematodes were reared on monoxenic plant tissue cultures. In this technique El Dorado alfalfa and Ladino white clover seeds were placed in wire baskets, scarified with concentrated sulfuric acid for 5 min, washed in running water for 30 min, and then treated with 2% chloramine-T for 4 hours. The seeds then were rinsed three times in sterile distilled water, soaked in 2,500 ppm streptomycin sulfate for 4 hours and placed in culture tubes containing 15 ml of modified Krusberg's substrate (Krusberg, 1961). Each culture tube containing 10-15 one-week-old seedlings received 200 irradiated nematodes; treatments usually contained 12 replicates. Clover cultures were harvested after 4 weeks while the alfalfa cultures were harvested after 6 weeks' growth; the nematodes were recovered by means of the Baermann funnel technique. Twenty to 25 alfalfa seedlings from each treatment were individually teased out under a dissecting microscope to collect eggs, larvae and adults for examination for morphological abnormalities. The progeny of irradiated *D. dipsaci* (alfalfa race) after the 6 weeks' harvest were re-inoculated onto El Dorado alfalfa tissue to trace the persistence of morphological mutation in subsequent generations. All nematode cultures were incubated at 23° ± 2°C.

After gamma irradiation the motility of fourth-stage larvae and adult *D. dipsaci* was measured under axenic conditions. The nematodes were suspended in sterile distilled water in B.P.I. dishes and observed after 6 hours, then daily for 7 days and again on the 21st day after irradiation. Each treatment was replicated five times with approximately a 200-nematode inoculum in each replicate.

Reaction of El Dorado alfalfa seedlings to irradiated *D. dipsaci* was evaluated by indexing the apices for the severity of swelling 4 weeks after inoculation. The symptom index consisted of five categories: 0 = no swelling in the shoot crown; 1 = slight; 2 = moderate; 3 = moderately heavy; and 4 = heavy.