THE INCREASE IN MALE ADULTS BY GAMMA-RAY IRRADIATION IN THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCognITA* CHITWOOD

BY

N. ISHIBASHI

Laboratory of Nematology, Central Agricultural Experiment Station, Konosu, Saitama, Japan

A gamma-ray irradiation of genital primordia before sexual differentiation increased the percentage of small male adults (0.7 mm body length) with one testis (early irradiation). On the 11th day after irradiation, the percentage of male larvae were 1.5%, 18.3% and 27.2% in the non-irradiated control, 10 kr and 20 kr dosages, respectively. The irradiation retarded the growth of nematodes in proportion to the dosage.

On the other hand, an irradiation at the time when the gonads were developing from cotyloid-shaped to V-shaped increased large males (approximately 1.5 mm body length) with 2 testes (late irradiation). Ten days later, the percentage of the males was 1.6% in the non-irradiated control, 24.9% and 33.5% in the 10 kr and 20 kr irradiation, respectively. The retardation of growth by this late irradiation was less than that by the early irradiation, and there was little difference in retardation between 10 kr and 20 kr dosages.

Since Steiner (1923) first reviewed intersex in nematodes including insect-parasitic, soil free-living and marine free-living forms, a number of studies have been made on sex in nematodes (Cobb, Steiner & Christie 1927; Christie 1929; Tyler 1933; Linford 1951; Ellenby 1954; Hirschmann & Sasser 1955; Hirschmann 1957). With the root-knot nematode, *Meloidogyne* spp., Van der Linde (1956) and Dropkin (1959) have shown that sex is determined by the environment and that the male to female ratio depends on the host. Triantaphyllou (1960) found in *M. incognita* and *M. javanica* that conditions unfavorable for the second stage, sexually undifferentiated, larvae induced an increment of male adults with one testis, and that, when a large number of the larvae entered and crowded in a host root, many of the second stage female larvae underwent sex reversal and then continued their development to adult males with two testes.

In the course of an investigation into the effect of ionizing radiations upon the reproduction of the root-knot nematode, *Meloidogyne incognita*, it was noticed that, when larvae were irradiated after entry into host plants, the number of male adults increased (Ishibashi & Taguchi 1965). This observation led to experiments designed to determine the stage at which irradiation is most critical for the nematode to be induced to males.

MATERIALS AND METHODS

The sweet-potato cuttings were cultured in full nutrient solutions and then transplanted into steam-sterilized sand when the roots were about 15 cm long.
When larvae of *Meloidogyne incognita* had hatched for not more than 24 hours, they were poured into the sand at a rate of about 5,000 individuals per plant. The plants were exposed to infection for 3 days at room temperature (20°-30° C). Then the plants were placed in nematode-free sand, and allowed to grow with full nutrients in a greenhouse at temperature between 20° and 35° C.

Before irradiation, the developmental stages of gonads were examined under phase contrast microscope; the roots were stained with boiling acid fuchsin lactophenol, transferred to pure lactophenol, and all nematodes were dissected out of the roots. This examination was started immediately after inoculation and continued every day thereafter until sex was completely differentiated (approximately 8 days after entry to host plants).

After the observations of gonad development, one group of the infected plants was irradiated immediately after the 3-day inoculation period and the other 6 days after the 3-day inoculation period. The infected plants were put between two sheets of acrylic chloride of 2 mm thick in such a way to obtain maximum concentration of the irradiation on the roots. The tops of plants were protected from irradiation with lead plates 7 mm thick. The irradiation was made by a Cobalt-60 source of 2,000 curies at a rate of 200 r per min. (in air), and the plants were rotated at 3¼ rpm during irradiation. The doses of irradiation were 10,000 and 20,000 r of gamma-rays. After irradiation, each plant was transferred to a Wagner pot containing about 2.5 kg steam-sterilized sand, and allowed to grow with full nutrients (Ishibashi, Kegasawa & Kunii, 1964) in a greenhouse at 25°-33° C. Each treatment consisted of five pots. On the 3rd, 5th, 8th and 10th or 11th day after irradiation, the root systems were stained with boiling 1% acid fuchsin lactophenol, and all nematodes were dissected out and mounted on slides in pure lactophenol for microscopic examination. Thus the gonad development was assessed and the growth curve of nematode body was determined by measuring the cross sectional area in square microns. The report of Triantaphyllou & Hirschmann (1960) was referred to for the morphology of gonad.

RESULTS

Immediately after the 3-day inoculation period, more than 90% of the second stage larvae had a globe-shaped genital primordium. But later the gonad development of larvae became less uniform in a population; 6 days after inoculation, 15% of the population had the globe-shaped genital primordium, 38% had a cotyloid-shaped, and 47% a V-shaped gonad.

I. Gamma-ray irradiation on infected roots immediately after a 3-day inoculation

The gonads of larvae were examined 3, 5, 8 and 11 days after irradiation. More than 100 larvae in each batch were observed under the microscope. Table I shows the gonad development expressed as percentage of each shape of gonad. The second stage larvae which had V-shaped gonads were apparently females, whereas larvae having L-shaped or cylinder-shaped gonads were assumed to develop into male