A Technique for Obtaining Quantities of Living *Meloidogyne* Females

R. S. HUSSEY[1]

Biochemical studies of plant-parasitic nematodes have been limited to a few species which can be cultured in large quantities. These are primarily species propagated monoxenically on callus tissue (4). Nematode populations recovered from these cultures consist of mixed stages, i.e. egg, larval and adult. Biochemical characters which might be age-dependent cannot be determined until methods are available for separating the stages.

Dropkin *et al.* (2) reported a procedure using commercial enzyme preparations to release adult female root-knot (*Meloidogyne* spp.) nematodes from galled roots. This procedure, however, did not allow for separation of the nematodes from the root debris. Dickson *et al.* (1) used a macerating technique but also did not develop a satisfactory separation procedure. The following is an extension of these macerating techniques which provides sufficient quantities of clean root-knot adult female nematodes for biochemical investigations.

Root-knot nematodes were propagated on tomato, *Lycopersicon esculentum* Mill. 'Rutgers' (1), grown in double-treated (steam and methyl bromide) sandy loam soil and coarse sand mixed 2:1. Twenty-five plants (15 cm tall) with well developed root systems were inoculated with 15 egg masses each and incubated in the greenhouse at 25–32 C. Plants were watered twice daily and fertilized biweekly with Ra-Pid-Gro® (Ra-Pid-Gro Corporation, Dansville, N. Y. 14437) fertilizer.

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[1] Research Associate, Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27607. Supported by Cooperative State Research Service USDA Grant No. 916-15-10.

Plants were harvested 45–50 days after inoculation and the root systems were excised and cleaned of debris. One hundred grams of cleaned roots were cut into approximately 2.5-cm sections and placed in one-liter Erlenmeyer flasks with 250 ml of a 50% solution of Pectinol 59L (a liquid pectinase concentrate, Rohm and Haas Co., Bristol, Pennsylvania) and agitated on a reciprocal shaker at 180 oscillations per min for approximately 10 hr at 25 C.

Macerated and softened roots were collected on a 20–60-mesh sieve series and washed with a high-pressure spray of tap water. The material collected on each screen was placed in separate beakers. Additional nematodes were dislodged from the softened root material collected on the 20-mesh screen by blending in a Waring blender in which the blades had been replaced with rubber tubing. This material was then screened as before.

Root debris and female nematodes were collected on a 60-mesh screen. Fractions (40–50 g) were placed in 250-ml glass centrifuge bottles, suspended in 150 ml of a 20% sucrose solution, and centrifuged at 1000*g* for 10 min.. Female nematodes (and some debris) were collected from the surface of the supernatant with a Pasteur pipet attached to a liter vacuum flask containing 300 ml of tap water. Nematodes were collected on the 60-mesh screen and transferred to a 250 or 150-ml beaker depending on the quantity of material. Most of the remaining debris was removed by washing with tap water and decantation. A stream of tap water was directed along the inside of the beaker to initiate a swirling motion. The

beaker was filled with swirling water and allowed to set for 30 sec. As nematodes settled in the center, most of the debris and some nematodes remained suspended. This suspension was poured onto a 60-mesh screen. This process was repeated until the only debris (mostly small galls) remaining was the same size as the female nematodes. Materials collected on the 60-mesh screen was reprocessed until most of the nematodes were recovered. Nematodes were placed in 1% sodium chloride and the final pieces of debris were removed with fine-pointed forceps under a dissecting scope.

Using this technique, 6 cc (approximately 7,000/cc) of clean nematodes (Fig. 1) were recovered from 25 severely infected tomato plants. The stains New Blue R (5) and potassium permanganate (3) were used to estimate the percentage of living nematodes. About 85–95% of the recovered female nematodes were living and in good condition. This technique provides a quantity of clean root-knot adult female nematodes suitable for biochemical studies.

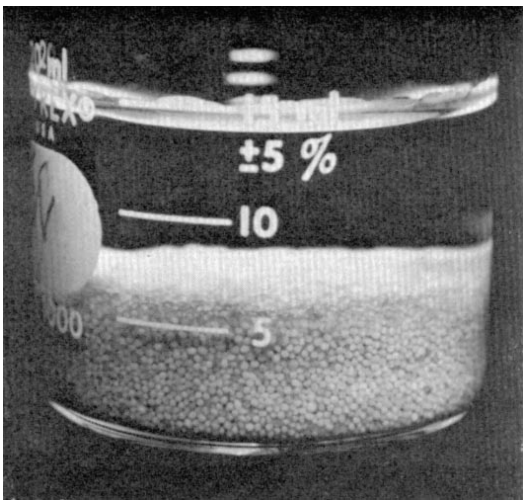

FIG. 1. Six cc of clean root-knot (*Meloidogyne incognita*) female nematodes.

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