INFECTION OF COTTON SEEDLINGS BY
MELOIDOGYNE INCognITA
AND A METHOD OF PRODUCING UNIFORMLY
INFECTED ROOT SEGMENTS

BY

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Larvae of Meloidogyne incognita failed to penetrate root tissues 4 cm from the root tip and only a few penetrated 2 cm from the tip. Following penetration, larvae initially became oriented acropetally, parallel to the stele and could migrate up to 12 mm towards the growing tip. However, by 32 hr they were randomly oriented. The inoculation procedure utilized assures uniform, synchronous penetration of cotton roots by these nematodes.

Infective larvae of root-knot nematodes, Meloidogyne spp., accumulate around the roots of host plants and generally penetrate near the apical meristem (Peacock, 1959). In many hosts, however, developing nematodes may be found not only in the tip, but along the entire axis of the root. This position could be explained by the sedentary nature of the feeding nematode and the ability of roots to continue growth following mild infections. However, Christie (1936) and Krusberg & Nielson (1958) reported that larvae of root-knot nematodes also can enter more mature tissues. Further, Minton (1962b) noted that migration of nematodes away from the tips of cotton roots and continued root growth resulted in nematodes becoming established in differentiating tissues. Migration of larvae within the root also has been postulated by Christie (1936).

As part of a program concerning the nature of resistance in cotton to M. incognita, it was necessary to devise a technique for the inoculation of cotton roots which would provide uniformly infected root-segments of comparable chronological and pathological development. It was important, therefore, to determine the ability of nematodes to penetrate older portions of the root and the extent of post-infection migration. This paper gives details of a method for obtaining infected cotton roots suitable for biochemical or histological studies, presents evidence for internal, acropetal migration of infective larvae, and provides evidence that larvae fail to penetrate mature root tissues.

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MATERIALS AND METHODS

Seed germination. Assemblies of special germination paper 1) were prepared, each assembly consisting of a single layer of autoclavable plastic wrap 2) and three layers of moistened paper, loosely rolled together with the plastic wrap outermost. Prior to use, the moistened rolls were placed on end in beakers of water and autoclaved at 15 psi for 15 mins. Seeds of cotton, Gossypium hirsutum L., were washed in running tap water for 30 mins and then immersed in 20% household bleach (1% sodium hypochlorite) for 20 mins. Residual bleach was removed by washing with sterile distilled water until the eluate was neutral to litmus paper. Treated seeds then were arranged in a single row between the second and uppermost layers of the autoclaved paper (Fig. 1A) and the entire assembly was re-rolled and again placed on end in a 250 ml beaker containing 100 ml of sterile distilled water (Fig. 1C). The plastic wrap added rigidity to the assembly, reduced water loss, and helped to protect the rolls from external microbial contamination. After 48 hr incubation in the dark at 29°C the seeds had germinated and the radicles had reached a length of 5-7 cm (Fig. 1B). Using this technique, germination was nearly 100% and the radicles were straight and white.

Inoculation. Washed and oven-sterilized vermiculite was moistened by shaking it in a plastic bag with sterile distilled water (1 liter H₂O to 1 kg vermiculite). The vermiculite then was uniformly packed into boxes similar to those described by Minton (1962a) but lined with plastic wrap 3). The wrap minimized desiccation of the vermiculite and wetting of the box. It was discarded after each test. Seedlings were transferred to the filled boxes and aligned approximately 1 cm apart, with their root tips extending 3-4 mm over the upper edge of a 2 cm-wide strip of Miracloth 4). A second strip of Miracloth was placed over the first, covering the root-tips (Fig. 1D). A suspension of second stage larvae, freshly hatched in Aretan (Crosse & Pitcher, 1952) was pipetted directly onto the upper strip. Preliminary studies showed that approximately 25% of the larvae applied would penetrate. Therefore, inoculation with 4 x the desired numbers of nematodes per seedling resulted in known levels of infection. Growth boxes were incubated in an environmental chamber at 28°C with an illumination intensity of 20,000 lux for 12 hr per day. Infected root segments located between the two layers of Miracloth were excised 24 hr after inoculation and the number of developing larvae determined after staining with acid fuchsin (Dropkin, Helgeson & Upper, 1969).

Penetration. The ability of M. incognita larvae to penetrate various portions of roots was tested in culture dishes by exposing limited areas of the radicle to nematodes. Individual seedlings, germinated in the paper assemblies were transferred to

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