A Technique for Studying Penetration of Roots of Plants by Endoparasitic Nematodes[1]

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A method for the study of penetration of roots by endoparasitic plant nematodes should: (i) have low variability so a reasonable number of replicates can be used in quantitative studies, (ii) provide conditions for a high rate of penetration so small amounts of inoculum can be used, (iii) provide material that can be readily fixed and stained for microscopy, (iv) retain moisture during incubation periods of several hours or days, (v) require little space and be convenient to place under different environmental conditions. These requirements are satisfied by the following technique.

It consists of incubating nematodes and seedlings between two 50 mm discs of Miracloth® in a 50 mm (I.D. bottom) petri dish. Miracloth is a coarse non-woven cellulose acetetate fabric. The desired number of seedlings are placed on the lower disc with the cotyledons and hypocotyl arranged vertically against the side of the dish. The second disc is placed over the roots and the nematodes, suspended in 1.0—1.5 ml of water, are added. The suspension of nematodes can be added before or after the second disc is in place without affecting results, provided air bubbles are not trapped between the discs. Difficulty with air bubbles is less when the suspension is added after both discs are in place. After the desired incubation a small amount of water is added, the top layer of Miracloth is removed, and the seedlings are lifted out, fixed, stained, cleared, and mounted for observation.

A higher percentage of penetration is usually obtained when the nematodes are added in 1.0 ml of water than in 1.5 ml of water. However, when a 1.0 ml suspension is used the Miracloth is not quite saturated, the system dries out rapidly, and root tips tend to grow into the Miracloth. The latter is the only annoying drawback we have encountered since care must be exercised in extricating root tips from the cloth.

When 1.5 ml suspensions are used to saturate the Miracloth, there is not enough excess water to seriously hamper penetration, and very few root tips penetrate the cloth. Incubation periods of 4 or 5 days can be maintained without adding water or resorting to the use of high humidity chambers. If more than 1.5 ml are added, penetration is drastically reduced because of excess liquid.

This technique has been used successfully with seedlings of alfalfa (*Medicago sativa* L.), red and ladino clovers (*Trifolium pratense* L. and *T. repens* L.), tomato (*Lycopersicon esculentum* Mill.), and tall fescue (*Festuca arundinacea* Schreb.) in various combinations with nematodes of the genera, *Pratylenchus, Heterodera,* and *Meloidogyne.* For example, 75–90% penetration occurred in 72-hr old alfalfa seedling roots during a 48-hr period of incubation when 25 females of *P. penetrans* were applied. When 10 females of *P. penetrans* were placed close to alfalfa seedling roots, penetration was 80–100%. Performance was quite consistent and 4 or 5 replicates were sufficient for significant results in most experiments.

This technique has distinct advantages

Received for publication 10 April 1969.

[1] Published with permission of the Director as Journal Article No. 69-11-43, University of Kentucky Agricultural Experiment Station.

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over similar methods employing agar, quartz, sand, soil, or filter paper. There are no particles or fibers that interfere seriously with microscopy. Rates of penetration were at least twice those obtained in sand and soil, and they were at least five times those obtained in filter paper and agar. Miracloth retained moisture much better than did filter paper on sand. In order to retain adequate moisture during a 4- or 5-day incubation period in dishes containing quartz sand (particle size: 85%, 840–420 μ; 12%, 420–250 μ; 3%, < 250 μ), it was necessary to use 25 g of sand and 4 ml of nematode suspension. This large volume of supporting medium lengthened the paths nematodes traveled to roots. It may not have provided optimal aeration. In Miracloth, path length and aeration appeared to be optimal for maximum activity of nematodes.