A Comparison of Two Methods of Synchronous Inoculation of Cotton Seedlings with *Meloidogyne incognita*

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Several methods of inoculating host plants with larvae of *Meloidogyne incognita* Chitwood have been developed. Chapman and Eason (1) enumerated desirable criteria for inoculating plants with endoparasitic nematodes. They introduced the use of Miracloth® to maintain moisture and provide a support medium for nematode penetration. McClure and Robertson (4) developed an inoculation method that provided uniformly invaded roots of comparable age and pathological development. Their technique utilized observation boxes, described by Minton (3), and incorporated two superimposed strips of Miracloth between which the cotton-root tips are inserted and upon which the inoculum is placed. By controlling the time and temperature of incubation and the width of the Miracloth strip, they accurately controlled the period of inoculation and thereby allowed synchrony in pathological development. Their method works well, but if used in large experiments it is time-consuming and expensive.

We report a "ragdoll" method of inoculation applicable to screening plants for resistance to *Meloidogyne* spp. and for obtaining large quantities of uniformly inoculated root segments for physiological studies. The ragdoll is constructed with two sheets of commercial seed-germination towel (30 x 45 cm), a half sheet of similarly sized waxed paper (23 x 30 cm), and two strips of Miracloth (2 x 45 cm). The germination towels are superimposed, moistened with distilled water, and steam-sterilized. Twenty-five surface-sterilized cotton seeds are arranged in a single row between the two sheets of germination towel. The seeded towels are rolled into a cylinder and wrapped with waxed paper to prevent desiccation. The seeded ragdolls are incubated in a beaker containing water about 10 cm deep until roots develop. When the seedlings are ready for inoculation with larvae, they are removed from the ragdoll and a strip of Miracloth is placed on the exposed germination towel. The seedlings are reset about 1 cm apart with their root tips extending 3-4 mm over the upper edge of the Miracloth strip. The second strip of Miracloth is superimposed over the first to cover the root tips. *M. incognita* larvae are applied onto the upper Miracloth in 0.1-ml aliquots/seedling. The ragdolls are rerolled and incubated in a beaker containing water. This method of inoculation was used for each of the following experiments.

In our first experiment (Fig. 1), we compared the ragdoll and McClure-Robertson (4) methods of inoculation with 'Deltapine 16' (root-knot susceptible) and 'Clevewilt' (compartively root-knot resistant) cultivars of cotton, *Gossypium hirsutum* L. The boxes and ragdolls were incubated in the dark at 30 C for 48 h to initiate seed germination. The seedlings were then inoculated with 200 freshly hatched *M. incognita* larvae/seedling and incubated in a growth chamber at 30 C with daily periods of 12 h light and 12 h dark. After 4 days, the root tissues between the Miracloth strips were removed and root segments were stained in

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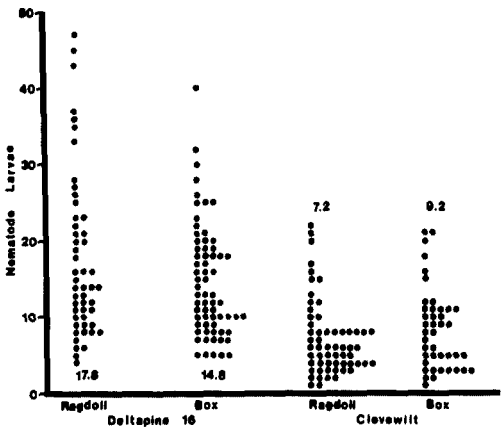

FIG. 1. Effects of inoculation method on penetration of a resistant and susceptible cotton by *Meloidogyne incognita* larvae in a 2-cm wide inoculation zone (means/method/cultivar as indicated). Each seedling was inoculated with 200 larvae. Each dot represents the root segment from one plant.

acid fuchsin-lactophenol and cleared with lactophenol. When the normality of the data curves for this experiment were analyzed by the Kolmogorov-Smirnov method (2), no significant differences were found between methods of inoculating either cultivar. Although larval penetration is reported to be about equal in root-knot susceptible and resistant cultivars (5), our data indicate that when measurements are made only in the zone of inoculation, i.e. the root segments between the Miracloth strips, there was more penetration in the susceptible than the resistant cultivar ($P = 0.05$).

In a second experiment in which the methods were again compared, larvae were counted in the entire root system rather than in segments in the inoculation zone. 'M8-cultivar' (susceptible) seedlings were inoculated with 100 larvae/seedling and harvested 1 and 6 days later. The average numbers of larvae penetrating roots after 24 h were 56.4 and 23.4 for ragdolls and boxes, respectively; 6 days after inoculation, the averages were 52.3 and 23.8 for ragdolls and boxes, respectively. About twice as many larvae penetrated roots inoculated by the ragdoll method than by the box method. The box method, however, had a more

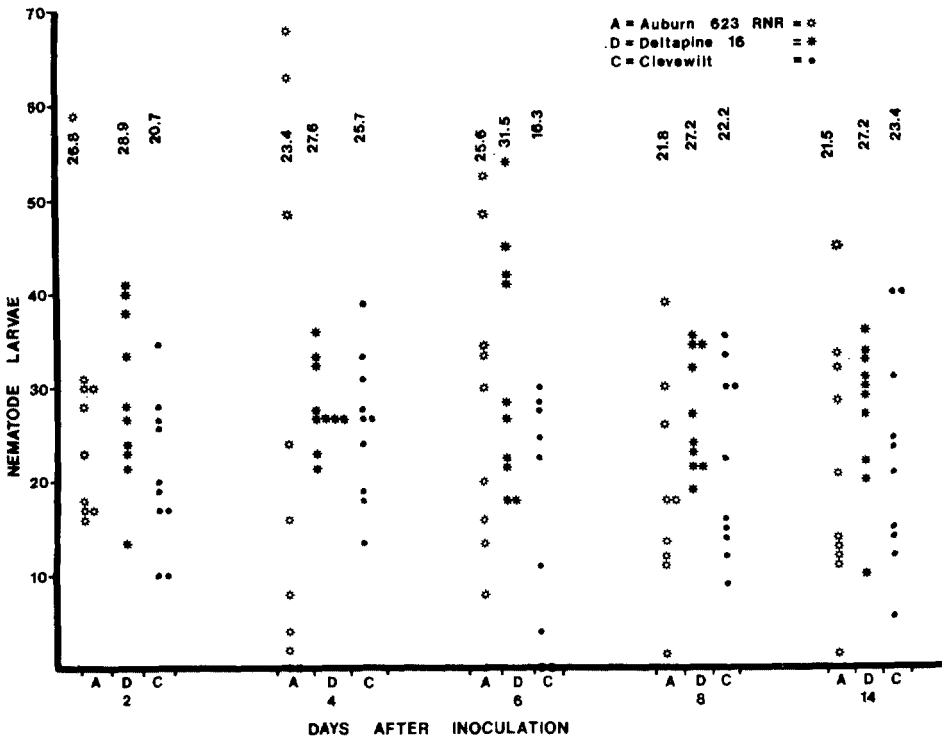

FIG. 2. Effects of inoculation method and susceptibility of cotton on penetration of *Meloidogyne incognita* larvae into entire root systems. Each plant inoculated with 100 larvae. Each dot represents one plant (means/per cultivar/time as indicated).

restricted range of the number of larval penetrations than the ragdoll method.

In a third experiment (Fig. 2), we determined the efficiency of the ragdoll method of inoculation by detecting varietal differences when the number of larval penetrations in the entire root systems were counted. 'Auburn 623' (very resistant), Clevewilt (moderately resistant), and Deltapine 16 (susceptible) seedlings were inoculated with 100 larvae/seedling by the ragdoll method and harvested 2, 4, 6, 8, and 14 days later. Larval counts on the entire root systems of the three cultivars confirm the conclusions of McClure et al. (5) that larvae penetration into roots is about equal in susceptible and resistant cottons. However, larval counts in the entire root system were markedly different from results of the first experiment in which counts only in the zone of inoculation was examined. We have no explanation for this discrepancy.

The ragdoll method of inoculating cotton seedlings gives results at least comparable to the McClure-Robertson (4) inoculation method and is more rapid, economical, and adaptable to larger numbers of seedlings. Removal of the root tissues beneath the Miracloth strips seems to be the best way of obtaining the most highly synchronized, infected tissues and it is recommended for physiological studies.

A disadvantage of the ragdoll method is the restriction of the time that seedlings can be grown. After the roots have grown about 15 cm, they become submerged in the water reservoir and turn black. This problem, however, is remedied by placing the ragdolls in an open-bottom support box and applying a slow-drip irrigation tube to the top of the ragdoll.

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