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Water Absorbent Polymer Aids in the Infestation of Field Sites with *Meloidogyne* Eggs[1]

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The root-knot nematode (*Meloidogyne* spp.) is one of the principal nematode pests of field and vegetable crops in the southern United States (6). *Meloidogyne* spp. are generally not uniformly distributed in naturally infested field sites, and sites frequently contain more than one species, thus complicating the evaluation of breeding lines for nematode resistance. To facilitate evaluation of nematicides, crop rotation schemes, resistance in plant introduction lines, or nematode damage to plants, a quick method of infesting field sites with inoculum is often necessary. A previous study described a method of applying *Meloidogyne* spp. eggs in a 0.125% agar suspension injected under pressure 10 cm below the seed row (1); however, with transplanted seedlings, such injection of eggs in the seed row infests a large soil volume where roots do not immediately grow. A technique was developed to artificially infest field sites with defined populations of *Meloidogyne* spp. by infesting transplant roots.

Inoculum was prepared by culturing *Meloidogyne* spp. on tomato (*Lycopersicon esculentum* cv. Rutgers) for 45–55 days in a greenhouse at 20–28 C. Nematode eggs were obtained by shaking infected tomato roots in 0.5% NaOCl for 4 minutes (4). The resulting egg suspension was collected on a 26-μm-pore sieve and immediately washed with water. Eggs were backwashed into a 0.125% agar solution or distilled water to which 6 g/liter Terra-sorb (Industrial Services International, Inc., Bradenton, Florida) was then added; Terra-sorb, a gelatinized starch hydrolized polyacrytonitrile copolymer, is a transplanting aid used to prevent desiccation of seedling roots. It has been shown to reduce transplant shock and wilting of tobacco seedlings, and tobacco transplant survival is improved when roots are dipped in a water slurry of Terra-sorb before transplanting (3).

Field test sites were fumigated with a single shank in-row application of 14 liter/ha ethylene dibromide applied 35 cm deep in the center of a 25-cm-high × 50-cm-wide bed 4 weeks before planting. Tobacco (*Nicotiana tabacum* L.) seedlings from methyl bromide-treated (37 g/m^2) beds were washed with tap water to remove soil and debris from the roots. Roots were inocu-

Table 1. Galling of tobacco roots grown in a field infested the preceding year by dipping seedling roots into an agar suspension of *Meloidogyne* spp. eggs.

Tobacco cultivar	Galling index*
Noninfested	
NC 95	0.6 a
Clemson PD 4	1.4 a
Infested†	
NC 95	5.7 b
Clemson PD 4	6.5 b

* Root galling scored on a 0–10 scale where 0 = no galls and 10 = 100% of roots galled. Means followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

† Field plots were infested the preceding year by dipping tobacco 'Clemson PD 4' seedling roots in a 1:1 *Meloidogyne arenaria* + *M. incognita* and agar slurry (500 eggs of each nematode/ml).

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TABLE 2. Galling of tobacco plants inoculated by dipping seedling roots in an agar and *Meloidogyne arenaria* egg slurry or a Terra-sorb and egg slurry.

Inoculum concentration (eggs/ml) and carrier medium*	Galling index†	
	Greenhouse	Field
0—distilled water	0	0
250—agar	5.0	
250—Terra-sorb	4.0	
500—agar	4.3	1.8
500—Terra-sorb	5.5	3.2
1,000—agar	6.0	2.4
1,000—Terra-sorb	6.0	4.1
2,000—agar	6.8	
2,000—Terra-sorb	6.8	
LSD at $P < 0.05$	2.7	1.3

* *M. arenaria* eggs were placed in a 0.125% agar solution or distilled water to which Terra-sorb (6 g/liter) was added.
† Gall index was based on a 0–10 rating scale where 0 = no galls and 10 = 100% of roots galled.

TABLE 3. Root galling on tobacco inoculated by dipping large bundles of seedling roots into a Terra-sorb and *Meloidogyne arenaria* egg suspension and transplanting into field plots.

Inoculum concentration* (eggs/ml)	Galling index†	Tobacco roots with galls‡ (%)
0	0 a	2.0
625	2.8 b	98.0
1,250	3.6 c	100.0

* *M. arenaria* eggs were washed into distilled water to which Terra-sorb (6 g/liter) was then added.
† Root galling scored on a 0–10 scale where 0 = no galls and 10 = 100% of root tissue galled 90 days after transplanting. Means followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.
‡ Twenty plants/100-plant plot were randomly selected and the roots rated for galling. Each treatment was replicated four times.

lated by dipping them into a slurry of agar and nematode eggs or a Terra-sorb and egg slurry. Excess inoculum was removed by gently shaking the roots. Seedlings were then machine transplanted with 60-cm plant spacing (5) using 1,870 liter/ha transplant water.

In a 1983 field trial, *M. incognita* race 3 and *M. arenaria* race 2 (6) were inoculated concomitantly on tobacco seedlings using the 0.125% agar root dip method. A randomized complete block design was used with four replicates. With an initial concentration of 500 eggs of each nematode/ml suspension, galling was observed following harvest only on inoculated plants. The following year 'NC 95' tobacco (resistant to *M. incognita* races 1 and 3) and 'Clemson PD 4' tobacco (susceptible to *M. incognita*) were planted into the same plots. Extensive galling was observed on both cultivars indicating that both nematode species became established following inoculation by the root-dip method (Table 1).

Terra-sorb was evaluated as a carrier medium for the *Meloidogyne arenaria* eggs in 1984. Eggs were placed in 0.125% agar solution or in Terra-sorb slurry at concentrations of 500 or 1,000 eggs/ml. Seedling roots were dipped individually into the egg slurries and then transplanted into 20-plant field plots with three replicates. Following harvest each plant was evaluated for root galling using a 0–10 rating scale where 0 = no galls and 10 = 100% of roots galled (2). Roots of a similar set of tobacco seedlings were dipped into the Terra-sorb or agar and egg slurries containing 250, 500, 1,000, or 2,000 eggs/ml and transplanted into 15-cm-d plastic pots filled with a Norfolk sandy loam soil-sand-peat mixture (2:1:1). A randomized complete block experimental design was used with four replicates. The plants were maintained in a greenhouse for 50 days, and the roots were then evaluated for galling. Both slurry preparations were suitable carriers for *Meloidogyne* eggs (Table 2). Use of Terra-sorb resulted in increased galling as compared to the agar in the field trial. Galling, however, was similar for the two slurries in the greenhouse. The moisture conserving properties of Terra-sorb may have enhanced egg survival in the field where conditions were very dry following planting in 1984.

Large bundles of tobacco transplants were dipped into a Terra-sorb and *M. arenaria* egg slurry and transplanted into field plots to evaluate the use of this inoculation method in evaluating breeding lines for *Meloidogyne* resistance. A randomized complete block design was used with four replicates. Twenty plants were selected at random from each 100-plant plot and rated for root galling (Table 3). Only 2% of plants

lacked galls at the low inoculum level, and all plants were galled at the high inoculum level.

The Terra-sorb and *Meloidogyne* egg slurry is easier to prepare than the agar slurry, and its use resulted in satisfactory infestation of several field sites with *Meloidogyne* spp. Terra-sorb slurries may have potential for application as carrier media for other organisms including parasites and pathogens of nematodes.

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