A Technique for Inoculating Field Sites with *Meloidogyne* Eggs

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Field study sites infested with *Meloidogyne* spp. are needed to evaluate the effect of these nematodes on crop growth for economic threshold studies (3,4) as well as for other biological studies. Since uniform, naturally infested test sites are difficult to locate, a procedure was developed to artifically infest fields with known levels of *Meloidogyne* eggs. The technique is a modification of a method developed to distribute corn rootworm eggs, *Diabrotica* spp., in field soils (6). Both procedures utilize a mechanized system which minimizes labor and produces a uniform infestation.

Eggs for inoculum were obtained by rearing *M. incognita* on tomato, *Lycopersicon esculentum* cv. Tropic, roots for approximately 90 days, washing roots free of soil, and collecting egg masses using an extraction technique similar to that of Byrd et al. (1). Since maximum viability and number of eggs was necessary, egg masses on root tissue were stirred for 5 min in 5% commercial bleach (0.25% NaOCl) to minimize physical and chemical damage. After stirring, the root and egg suspension was passed through a 170-mesh sieve to remove root debris and then through a 500-mesh sieve to recover individual *M. incognita* eggs. Eggs were rinsed thoroughly with water on the 500-mesh sieve to remove residual bleach.

Eggs of *M. incognita* were backwashed from the 500-mesh sieve into a 0.125% agar solution and thoroughly mixed. This suspension reduced settling and facilitated a uniform distribution of the eggs in a liquid carrier medium. Palmer et al. (5) utilized this procedure to suspend *D. virgifera* eggs to artifically infest corn at time of planting. In this suspension, *Meloidogyne* eggs remain evenly distributed for several hours (Table 1). Egg hatch in 0.125% agar, 22.1%

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Table 1. Settling of *Meloidogyne incognita* eggs in 0.125% agar solution and in water.

Settling time (minutes)	Number of eggs/ml collected at various depths by pipet sample			
	Agar—Eggs		Water—Eggs	
	2 cm	7 cm	2 cm	7 cm
0	214b	200b	270b	372a
40	228b	205b	43c	41c
75	246b	211b	7c	12c
105	198b	202b	—	—
240	200b	270b	—	—

Values followed by a letter in common are not significantly different ($P = <0.05$) according to Duncan's multiple-range test.

±5.6, was not significantly different from egg hatch in water, 19.4% ±9.0.

The egg suspension was placed in a 18.9-liter stainless steel canister pressurized to approximately 1.4 Kg/cm² with bottled, compressed air. A pressure regulator on the air cylinder controlled pressure in the stainless steel canister and a ball valve allowed flow control of the egg suspension. The pressurized liquid was forced through 6.4-mm-i.d. vinyl tubing and directed behind fumigation shanks attached to a tractor tool bar. Injection shanks were run about 10 cm deep directly under the seed row. Seed was planted over this band of inoculum within 24 h and then followed by irrigation. Known amounts of *M. incognita* eggs were applied to the soil by calibrating canister pressure and ground speed as well as the egg concentration in the agar suspension.

In a field trial during spring 1980, differential levels of inoculum were applied to obtain various levels of infection. To evaluate the effectiveness and uniformity of this artifical inoculation technique, 20 plants from each plot were rated for root galling at time of harvest. A 0–8 rating scale was used and tranformed to a 0–100 scale (2). The rate of increase of gall index relative to inoculum density apparently decreased at higher inoculum densities (Fig. 1). We speculate that the nonlinear relationship between inoculum density and gall index is indicative of multiple penetrations or increased competition for available feeding sites at the higher densities (7). It would also be possible to satisfactorily describe the relationship with a linear function, but with a somewhat lower r^2. The gall index was closely related to the calculated number of eggs deposited per foot of row ($r^2 = 0.76$) by the selected model, suggesting that the technique resulted in uniform infestations of *M. incognita* on tomato roots in most plots of this trial.

Tomato plantings on this site during the 1981 growing season were heavily infested with *M. incognita*, indicating that a population had been established from the previous year's inoculation.

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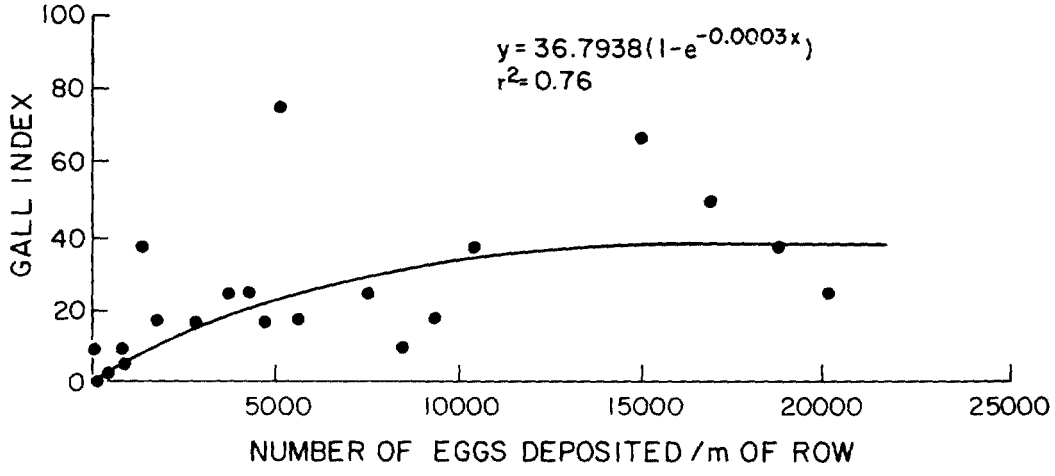

Fig. 1. Relationship between number of eggs deposited per meter, using a mechanical inoculation system, and a gall index.

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