

A Method for Field Infestation with *Meloidogyne incognita*

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Abstract: A field inoculation method was developed to produce *Meloidogyne* spp. infestation sites with minimal quantities of nematode inoculum and with a reduced labor requirement compared to previous techniques. In a pre-season-methyl bromide-fumigated site, nematode egg suspensions were delivered at concentrations of 0 or 10^x eggs/m of row where $x = 2.12, 2.82, 3.52,$ or 4.22 through a drip line attached to the seed firmer of a commercial 2-row planter into the open seed furrow while planting cowpea. These treatments were compared to a hand-inoculated treatment, in which $10^{3.1}$ eggs were delivered every 30 cm in 5 ml of water agar suspension 2 weeks after planting. Ten weeks after planting, infection of cowpea roots was measured by gall rating and gall counts on cowpea roots. A linear relationship between the inoculation levels and nematode-induced galls was found. At this time, the amount of galling per root system in the hand-inoculated treatment was less than in the machine-applied treatments. Advantages of this new technique include application uniformity and low population level requisite for establishing the nematode. This method has potential in field-testing of *Meloidogyne* spp. management strategies by providing uniform infestation of test sites at planting time.

Key words: cowpea, infestation levels, infestation method, *Meloidogyne incognita*, nematode inoculum, root knot nematode, technique, uniform field infestation.

Field studies of management and ecology of plant-parasitic nematodes require uniformly distributed, medium-to-high population levels of the test nematode. In naturally infested commercial fields such conditions rarely occur (Goodell and Ferris, 1980), and large horizontal variations in population levels may be present. This can cause strong covariate effects that may confound experimental results. At the same time, non-target nematodes with host ranges similar to those of the test nematode may be present and confound experimental results. For example, *Pratylenchus neglectus* was favored in cereal rotations with a high frequency of *Heterodera avenae*-resistant cereals whereas the *H. avenae* population density was suppressed (Rivoal et al., 1995). Several methods have been proposed to achieve a uniformly infested test site. One technique uses transplants infested in the greenhouse or dipped in egg suspension amended with a water-absorbent polymer as carriers of plant-parasitic nematodes into field plots (Bird et al., 1978; Fortnum et al., 1987). A second technique employs commercial shanks, similar to those used for dispensing fumigant or fertilizer, to apply nematode egg suspensions 10 cm below the future seed row of a susceptible crop, or with a commercial planter when nematode eggs were imbedded in calcium alginate in a trip before planting (Ball and Ferris, 1982; Koenning and Barker, 1992). A third technique uses drip irrigation for delivery (Becker et al., 1989). A common

fourth method is the application of nematode egg suspensions into a stand of a susceptible host crop. All techniques have limitations related to infestation uniformity or labor-intensity requirements.

The objective of our current study was to test a simple method designed to achieve uniform infestation with *Meloidogyne incognita* over an extended area. The test area was pre-season-fumigated with methyl bromide to reduce possible side effects of soil-borne pathogens or other plant-parasitic nematodes.

MATERIALS AND METHODS

The trial was conducted on a silt loam soil (18% sand, 60% silt, 22% clay, pH 6.9) following a wheat crop on the Meigs Research Farm at Purdue University, West Lafayette, Indiana. Soil was prepared with a chisel plow 30 cm deep and a field cultivator before soil fumigation. The entire experimental area was fumigated with methyl bromide (98% methyl bromide and 2% chloropicrin) at 450 kg/ha by a commercial applicator on 9 June 2004 following pesticide label recommendations, leaving the test site of 30 × 80 m tarped with high barrier 0.025-mm-thick polyethylene sheeting (Cadillac Products Inc., Paris, IL). Six days after application, the polyethylene tarp was cut and removed 24 hours later. On 23 June, the entire test area was cultivated at a 7 to 10-cm depth and then planted to cowpea (*Vigna unguiculata* cv. Chinese red). *Meloidogyne incognita* had been previously raised on tomato (*Lycopersicon esculentum* cv. Rutgers) for 3 to 4 months. The population was originally isolated as a single egg mass from watermelon roots grown in the soil from a field near Vincennes, Indiana. Nematode eggs were harvested using the NaOCl method (Hussey and Barker, 1973). Egg concentrations were adjusted to deliver 0 or 10^x eggs/m of row where $x = 2.12, 2.82, 3.52,$ or 4.22 in 7.2 ml of 0.125% agar. Egg suspensions were delivered via a CO₂-pressurized system (R&D Sprayers, Opelousas, LA) while planting rhizobium-inoculated (label rate of Nitragin, Nitragin Inc., Milwaukee, WI) cowpea at 33

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seeds/m of row in rows in East-West direction at 75-cm center-to-center spacing with a 2-row commercial planter. A delivery system was fabricated from 0.6-cm OD polyethylene tubing to dispense nematode egg suspensions through a commercial drip tubing assembly at the distal ends of the commercial seed firmers of both planter rows. Dispensing rate was regulated with an in-line installed flow regulator assembly with an orifice plate with a 950- μ m-diam. opening (no. 35; Teejet, Wheaton, IL). At the two highest egg concentrations a 500- μ m opening screen was used. Treatments were arranged as 33-m-long passes, side-by-side treatments in a randomized complete-block design with four replicates. Aliquots of the egg suspensions were collected after passage through the applicator and incubated on modified Baermann funnels at 28 °C for 4 days, similar to the one described by Hooper (1986). The eggs had an average hatch rate of 12%. The freshly planted rows were irrigated through commercial drip lines delivering 4.5 liter water/m of row. On 2 July a sixth treatment received 5 ml of 250 eggs/ml of freshly prepared and 0.125% agar-amended nematode egg suspension from greenhouse cultures. This suspension was hand-inoculated at 5-cm depth with a pipet every 30 cm in the row of the cowpea seedlings, delivering about $10^{3.1}$ eggs/m of row.

On 31 August the plots were divided into three sections lengthwise, and thus sample locations were assigned in a split-block design. In each section across the plots, cowpea root systems from 30-cm strips were lifted from soil using a spade. Plants were transported to the laboratory and nematode-induced galls were counted. Plant top dry weights and root fresh weights were determined. Roots were rated for galling severity following a scale from 0 to 9 (0 = no galling, 9 = severely galled) similar to the one proposed by Bridge and Page (1980) and nematode-induced galls were counted. During the season, the cowpea crop received nitrogen fertilizer.

After transformation to improve homogeneity (\log_{10} -transformation for gall counts, $\arcsine[\sqrt{(\text{gall rating}/9)}]$ -transformation for the rating data), data were subjected to an analysis of variance with inoculation level as main factor and the sampling location as the split-block factor using PROC GLM in SAS (SAS Institute, Cary, NC). Treatment effects were partitioned into linear, quadratic, cubic, and quartic contrasts using coefficients based on $\log_{10}(x+1)$ -transformed infestation levels. Significant contrasts were then explored for the significant contrasts as regressions using PROC REG in SAS.

RESULTS AND DISCUSSION

With this technique we achieved a uniform nematode distribution. Galling response of the cowpea roots was similar at the different strips within each of the infestation levels of the machine delivery (Fig. 1). Root fresh weight, gall rating, and gall counts were not sig-

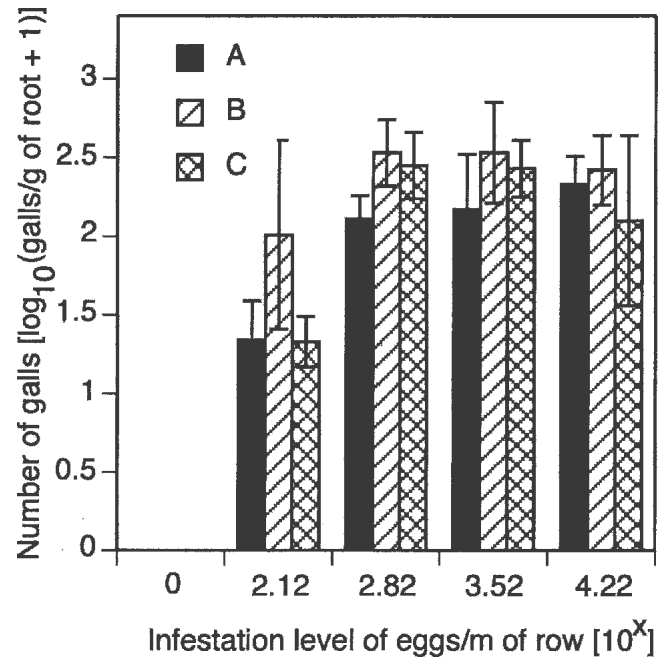


FIG. 1. Number of galls induced by *Meloidogyne incognita* per gram root fresh weight [$\log_{10}(\text{galls/g of root} + 1)$] at three strips along cowpea rows: A) Eastern strip of the rows. B) Center strip of the rows. C) Western strip of the rows. Strip locations in the rows had no significant effect within the different infestation levels. "0" indicates the non-infested control.

nificantly different among the sampling split blocks. Accordingly, plot means were used for the regression analysis for the machine-applied inoculum levels only. Root fresh weights and galling severity followed a linear regression. Root weights increased 0.09-fold with increasing inoculum levels (Fig. 2A), which was probably due to the increased root mass of the nematode-induced galls ($r^2 = 0.79$, $P \leq 0.05$). The nematode-induced galling severity increased 0.17-fold with increasing inoculum levels ($r^2 = 0.96$, $P \leq 0.01$; Fig. 2B). The gall counts were related to the inoculum levels in a quadratic relationship ($r^2 = 0.97$, $P \leq 0.05$; Fig. 2C), suggesting a type of saturation curve, which seemed appropriate because cowpea plants at the highest infestation level were severely compromised in growth and died (data not shown). Overall, shoot weight of cowpea was suppressed, possibly resulting from the lack of beneficial microorganisms in the recently fumigated soil. All machine-applied inoculum levels had higher gall ratings (lowest level: 0.36 in arcsine-transformed format) than the hand-inoculated treatment (0.22 in arcsine-transformed format). The gall counts per root weight in the hand-inoculated (1.16 of the log-transformed counts) treatment were lower than any of the machine-inoculated treatments (lowest machine-inoculated: 1.56, $P \leq 0.05$). This may have been partially based on the reduced time for nematode reproduction in this treatment compared to the co-application of seed and nematode inoculum at planting. The fumigation-suppressed *H. glycines* pre-

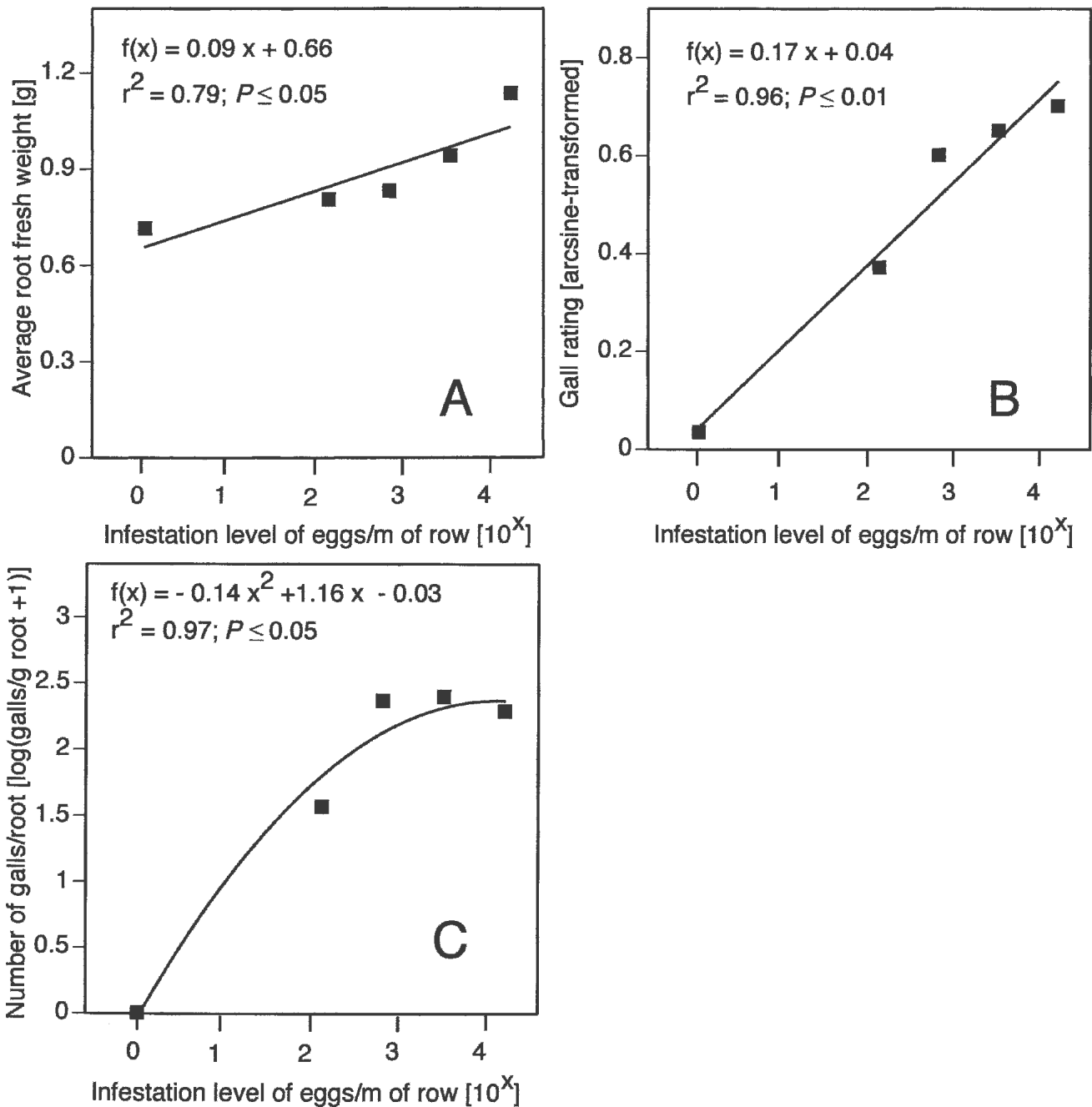


FIG. 2. Response of cowpea after infestation with *Meloidogyne incognita* during planting. A) Average root fresh weight [g]. B) Severity of *Meloidogyne incognita*-induced galling [arcsine-transformed data of rating on a scale 0–9]. C) Number of galls induced by *Meloidogyne incognita* per gram root fresh weight [$\log_{10}(\text{galls/g of root} + 1)$]. “0” indicates the non-infested control.

season populations in this field (5.8 cysts with 36 eggs/100 cm³ soil) did not appear to have had any detectable effect on the experimental results, and no reproduction of *H. glycines* was detected.

In summary, this new technique has benefit in the need for reduced inoculum for infestation and improved uniformity and reliability of successful nematode nursery establishment. The inoculum was distributed uniformly and required only limited preparation time before one trip across the field allowed the co-application of planting and nematode delivery. For ex-

ample, this technique will be effective in planting field screens for *Meloidogyne* spp. resistance into field sites when no naturally, uniformly infested sites are available.

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