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A Novel Technique for Infesting Field Sites with Encapsulated Eggs of *Meloidogyne* spp.[1]

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Abstract: Eggs of *Meloidogyne arenaria* race 1 were encapsulated in calcium alginate for use as inoculum to infest peanut field plots. Some eggs within the capsules remained viable up to 10 weeks after preparation. A field site was successfully infested at peanut planting and (or) 6 weeks later. Dual applications of nematode inoculum (at planting and 6 weeks later) were superior to single applications (at planting or 6 weeks after planting). Field-site infestation levels at the end of the first year were related to the amount of inoculum dispersed and timing of the infestation ($P = 0.001$). Peanut yield was only slightly affected in the first year, but significant ($P = 0.02$) yield suppression occurred during the second year after field infestations. The negative relationship between the numbers of *M. arenaria* eggs and juveniles per 500 cm^3 soil in the fall and the percentage of peanut hull galled the second year was described by a quadratic model ($P = 0.002$, $R^2 = 0.41$).

Key words: Arachis hypogaea, damage threshold, infestation technique, *Meloidogyne arenaria,* nematode, peanut, root-knot nematode.

Research on plant-parasitic nematodes at field sites provides a diversity of challenges to the researcher. Foremost of these challenges is the selection of a site that is suitable in terms of edaphic properties as well as the presence and spatial patterns of the nematode species to be studied. Evaluation of chemical or biological control agents or plant resistance to nematodes requires uniform population densities of these pathogens. A range of population densities is necessary in experiments designed to establish nematode-damage thresholds or ecological studies evaluating density-dependent factors (2).

Several techniques for infesting fields with nematodes have been reported. Introduction through a drip-irrigation system did produce uniformly infested sites (3). The use of tobacco transplants dipped in a polymer solution containing eggs of *Meloidogyne* spp. was effective in other research (7). An agar suspension containing eggs of *Meloidogyne incognita* (Kofoid & White) Chitwood was successfully used to establish this nematode at various population densities (1). Recently, several authors have reported encapsulation of nematodes in calcium alginate gels (9), and kappa carrageenan has shown promise (6). The current research was initiated to evaluate the use of calcium alginate as a medium for encapsulating plant-parasitic nematodes for distribution in field sites to establish various population densities of *Meloidogyne arenaria* (Neal) Chitwood race 1.

Methods and Materials

Eggs of *M. arenaria* race 1 on ca. 12-week-old infected tomato (*Lycopersicum esculentum* Mill. cv. Tropic) were collected by the NaOCl method (8). The eggs were encapsulated in calcium alginate (9) (Protan Inc., 777 Larksielt Road, Commack, NY 11725), with calcium gluconate as a complexing agent. Pellets were stored in 100 mM calcium gluconate overnight, rinsed, and stored in tap water at 10 C in a refrigerator. Pellets were prepared 22–25 May 1989 for use at peanut (*Arachis hypogaea* L.) planting on 26 May 1989. Peanut seed were inoculated with a commercial preparation of *Bradyrhizobium* sp. (*arachis*) Jordan before planting. A second preparation of pellets was made 10–13 July 1989 for a side-dress treatment. On each occasion, inoculum was quantified by determining the average weight of pellets and then press-

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ing individual pellets between two microscope slides to enumerate eggs and juveniles. Each pellet contained ca. 122 eggs and juveniles ± 12 (0.0375 g/pellet) on 26 May and ca. 139 ± 20 eggs and juveniles (0.0330 g/pellet) on 14 July 1989. Surface moisture was removed from pellets with paper towels immediately before dispensing pellets into the planter hopper.

Pellets were placed 4-cm-deep in the rows with a cone planter, and peanut cv. Florigiant was planted over the pellets the same day. Selected plots received a second infestation treatment 6 weeks later by placing pellets approximately 4 cm deep and 7–8 cm away from the plants on the side of the row, using the cone planter. Pellets from each preparation date were stored in tap water at 10 C in a refrigerator. Infectivity of nematodes in stored pellets was evaluated 3 and 10 weeks after the preparation date in a greenhouse bioassay on tomato cv. Rutgers. The bioassay was a factorial design with two ages of pellets and nine inoculum levels. Pellets were added to soil (a greenhouse mix of sand and loamy sand; 80% sand, 15% silt, 5% clay) in 15-cm-d clay pots at rates of 0, 1, 2, 4, 8, 16, 32, 64, and 128 pellets/pot. A tomato plant was placed in each pot on top of the pellets and covered with soil. The numbers of galls per root system were determined 28 days after inoculation.

Five inoculum treatments and a control were utilized in the field study. Treatments were 3,035 eggs per meter of row at planting; 6,070 eggs per meter of row at planting; 3,035 eggs per meter of row at planting and again 6 weeks after planting; and 6,070 eggs per meter of row at planting plus 3,035 eggs per meter of row 6 weeks after planting. One treatment received only a side-dress application of 3,035 eggs per meter of row 6 weeks after planting. The soil was a Fuquay sand (93% sand, 5% silt, 2% clay; 0.6% organic matter, pH 6.0). Plots were four rows 6.1 m long spaced 0.97 m apart with 1.5-m alleys between plots. Plots were arranged in a randomized complete block design with six replications. Peanuts were treated according to standard peanut management practices both years.

After peanut harvest, the field was disked and planted with wheat during the winter of 1989–1990. The wheat was disked under in the spring of 1990, and peanut cv. NC7 was planted. Rows were located as closely as possible (within 30 cm) to the previous year's rows. Soil samples for nematode assay were collected from the center two rows of each plot. Samples consisted of twelve 2.5-cm-d cores taken to a depth of 20 cm. Soil cores were composited, and 500 cm^3 of soil were processed by elutriation and centrifugation (5) to extract juveniles. Roots were collected from the elutriator and processed to extract eggs (4). Soil samples were collected preplant and immediately prior to peanut harvesting each year. Fresh pod weights were determined each year. Additionally, peanuts were rated for the percentage of galling and necrosis of pods each year (0–100 scale).

Data were subjected to analysis of variance, and the Waller-Duncan k-ratio *t*-test was used to separate means. Nematode data was transformed ($\log_{10}[x + 1]$) to standardize the variance. Regression analysis was used to relate log-transformed nematode numbers to peanut yield and gall indices.

Results

Bioassay of pellets (ages 3 and 10 weeks) on tomato revealed that some loss of nematode infectivity occurred during storage; pellet age as well as pellet number affected the resulting numbers of galls on tomato transplants ($P = 0.0001$). The number of galls on tomato for 10-week-old pellets averaged over all inoculum levels was 155 ± 104 versus 262 ± 153 for pellets aged 3 weeks. Even though there was some loss of viability over time, sufficient numbers of viable nematodes remained such that inoculum could be collected and stored over a period of several weeks before its application.

Field infestation of peanut plots was ef-

fective at both application dates (Table 1). Single applications at planting resulted in approximately 1,000–2,000 eggs/500 cm^3 soil, whereas a second application resulted in approximately 3,500 eggs and juveniles/500 cm^3 soil at peanut harvest. A single application of pellets 6 weeks after planting resulted in an average of 364 eggs and juveniles/500 cm^3 soil at peanut harvest. Gall indices on peanut pods in 1989 were affected by the amount of inoculum applied ($P = 0.01$) (Table 1). Numbers of *M. arenaria* juveniles per 500 cm^3 soil were generally below detectable levels in the spring of 1990 prior to peanut planting. Final levels of *M. arenaria* eggs and juveniles in 1990 were affected little by the previous year's treatments. Plants in control plots exhibited only traces of this pathogen.

Gall indices and peanut yield in 1990 were regressed against *M. arenaria* population density from the fall of 1989. The gall indices on peanut pods in 1990 were adequately described by a quadratic model where gall indices = $0.3026 + 3.36(\log_{10}[x + 1]) - 0.144(\log_{10}[x + 1])^2$; ($P = 0.002$, $R^2 = 0.41$). Peanut yield was not altered greatly as a result of nematode treatments in 1989 (Table 1). Yield of peanut cv. NC7 was suppressed by more than 30% by the high population density relative to the control in 1990. The relationship between population density (from the fall of 1989) with peanut yield also was described by a quadratic model where peanut yield = $6{,}803 + 11.57(\log_{10}[x + 1]) - 59.61(\log_{10}[x + 1])^2$, ($P = 0.02$, $R^2 = 0.21$).

Discussion

This method of infesting field plots provides several advantages over previously described methods. Inoculum can be collected and prepared as long as a month before application. The technique can be used to provide a uniform nematode distribution useful to plant breeders or relatively discrete but varying population densities for other purposes. The pellet carrier allows for the exact placement and maximum effectiveness of the inoculum. Dual applications would be best if high population densities are desired, but different numbers of eggs at different times provided an adequate range of densities for evaluating the damage potential of *M. arenaria* on peanut. Improvements in this method might be the use of kappa carrageenan capsules, which can be dried and stored for long periods of time. This material would also facilitate the use of mechanical planters. Pellets could be placed

Table 1. Influence of infestation treatments with encapsulated eggs and juveniles of *Meloidogyne arenaria* race 1 on final population density, peanut yield, and peanut pod gall index in 1989 and 1990.

Inoculum level/m of row		Final densities of *M. arenaria* (eggs and juveniles/500 cm^3 soil in 1,000s)				Fresh pod weight (kg/plot)				Gall index (0–100%)			
		1989		1990		1989		1990‡		1989		1990	
At planting	6 weeks after planting	Mean†	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	0	0.011 c	0.02	0.023 c	0.85	5.5 ab	0.50	6.6	0.89	0 b	0	0 c	0
3,035	0	1.2 ab	6.9	18.2 ab	24.7	5.4 ab	0.67	5.2	1.7	4.3 b	3.7	8.5 ab	5.7
6,070	0	1.4 ab	2.4	24.9 a	17.0	5.6 ab	0.67	5.9	1.1	15.0 a	4.5	8.3 ab	4.1
3,035	3,035	3.5 a	6.2	31.9 a	30.2	5.3 ab	0.65	6.2	1.3	5.2 b	5.0	11.2 a	6.3
6,070	3,035	3.7 a	3.7	18.0 ab	18.6	5.1 b	7.2	4.5	2.1	20.0 a	5.5	12.8 a	4.7
0	3,035	0.36 b	3.9	25.3 ab	13.5	4.9 c	7.5	5.7	0.95	5.3 b	9.6	5.3 b	3.8

All data are means of six replications; means followed by the same letter are not significantly different by the Waller-Duncan k-ratio *t*-test (k-ratio = 100).

† Means of nematode data are antilogs of transformed ($\log_{10}[x + 1]$) data, standard deviation (SD) is of untransformed data.

‡ The relationships between 1990 peanut yield and gall indices and the 1989 final population density were described by a quadratic model ($P = 0.05$).

in the seed hopper box and dispensed exactly on the rows with a tractor-mounted planter as was done in our experiment. The same planter could be used to plant the susceptible crop into the same location where nematodes are placed.

Placement of plots such that the second year's rows corresponded exactly with the first year's rows was desirable for this research, but not essential. Tillage operations for weed control and seed bed preparation results in considerable mixing of soil and inoculum within a plot. Disking, however, results in little net soil movement. Data collection from the center two rows of the four-row plots was adequate compensation for any soil movement, as relatively little contamination of control plots occurred. The spatial dispersal of the nematodes, as a result of land preparation, may explain the almost undetectable levels of *M. arenaria* encountered in the spring of 1990. Overwinter survival of *M. arenaria* was at relatively high population densities in microplot research (10). This discrepancy between microplot versus field data indicates a need for further research.

Our use of peanut as an experimental crop represents a rigorous evaluation of this infestation technique. Peanut is a poor crop for increasing inoculum density because most roots and shoots are dug and air dried at harvest. This practice results in the destruction of much inoculum within the field site. The growth of a susceptible winter cover crop such as ladino clover should result in much higher inoculum densities at the beginning of the next season. The slight damage to peanut during the first year of our experiment indicates that a two- to three-fold increase in the amount of inoculum at planting could effect higher densities for the subsequent year. These data confirm other work in field and microplot research by verifying the damage potential of *M. arenaria* on peanut in North Carolina (10). Further modification and improvements in this technique should provide a valuable tool for researchers in the future.

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