RESEARCH NOTES

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Usefulness of Egg Assays in Nematode Population-Density Determinations[1]

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Much progress has been made in developing techniques for characterizing population dynamics of nematodes. Nevertheless, most assay procedures have numerous inherent limitations (1,13). The efficiency of given methods may be dependent on soil type, moisture, and other environmental and biological factors (1,13). Certain extraction methods (e.g., Baermann funnel) depend on nematode motility. In addition, the motility of juveniles of nematodes such as *Meloidogyne javanica* (Treub) Chitwood may not be correlated with infectivity (12). These problems can be overcome sometimes through bioassays (1,8,10).

The precision in detecting changes in nematode populations over time can be enhanced greatly by determining egg numbers along with other life stages of these organisms (11). Numbers of eggs in cysts of *Globodera rostochiensis* (Wollenweber) Behrens have long been utilized in the study of this important pathogen (9). The same method is also suitable for *Heterodera* spp. Procedures for egg assays of *Meloidogyne* species and other nematodes have been developed more recently (1–4,6). Eggs of ectoparasitic nematodes and migratory endoparasites are more difficult to assay and may have to hatch before they can be identified (1,5). Eggs of *Meloidogyne* species or other taxa that form eggs in masses can be assayed by NaOCl extraction techniques (3). This approach is particularly useful in evaluating resistant cultivars, breeding lines, and nematicides (1). These techniques, however, currently are employed in only a limited number of nematological laboratories.

This report presents data from typical experiments on *Meloidogyne* and *Heterodera* species and depicts the importance of monitoring their egg populations. The data came from microplot and field tests involving nematicide evaluations and (or) cultural practices including subsoiling and rotation.

Establishment of experiments: Microplots established with fiberglass borders in a Norfolk loamy sand (87% sand, 9% silt, 4% clay, pH 6.8) in North Carolina were fumigated with methyl bromide at 0.14 kg/m^2. Each plot was infested with *Meloidogyne* spp., with the levels indicated in Tables 1 and 2, and the mycorrhizal fungus *Glomus macrocarpus* (ca. 2,000–5,000 spores/plot). Test crops were peanuts (*Arachis hypogaea* L.) and tobacco (*Nicotiana tabacum* L.).

Field experiments with *Meloidogyne* species were designed to test the efficacy of aldicarb in controlling *M. incognita* (Kofoid and White) Chitwood on cotton (two tests in Brazos County, Texas) and *M. arenaria* (Neal) Chitwood on peanut (one test in Eastland County, Texas) (Table 3). All three tests were conducted on loamy sand soils (85% sand, 7% silt, 8% clay, pH 7.5

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TABLE 1. Numbers of *Meloidogyne hapla* eggs and juveniles recovered from peanut in microplots treated with different nematicides in North Carolina.

Nematicide treatment (kg a.i./ha)	Nematode numbers per 500 cm³ of soil							
	Midseason				At harvest			
	Eggs	CV	Juveniles	CV	Eggs	CV	Juveniles	CV
Compound I								
0	8,600*	112	600*	139	2,770*	65	2,670*	49
3.4	300†	143	100†	107	250	167	1,100†	127
10.1	0		0		700	245	10	160
Compound II								
0	4,900*	212	200*	124	28,100*	160	28,000*	116
3.4	200	245	40	221	600	153	500	120
10.1	0		0		6,900	221	100	213
Compound III								
0	4,900	104	100	99	17,000	83	14,200*	46
2.2	5,700	110	300	129	62,800	148	34,200*	44
4.5	9,100	129	300	142	65,000	141	42,400	67

Statistical differences at $P \leq 0.05$ level of significance for compound III versus I and II.
* Statistical differences at $P \leq 0.05$ level of significance for control vs. average of low and high chemical rates.
† Statistical differences at $P \leq 0.05$ level of significance for low vs. high chemical rate. (Note: Last two contrasts calculated for each chemical, separately.) Nematode numbers are means of seven replicates; $\log_{10}(X + 1)$ transformation was used for the statistical analyses; initial inoculum level was 10,000 eggs per 500 cm³ of soil.

in Brazos County; and 88% sand, 8% silt, 4% clay, pH 7.8 in Eastland County). Plots were two rows, 1 m apart and 24 m long.

For the *Meloidogyne* spp. in microplot and field experiments, composite soil samples (15–20 cores 2.5-cm-d) were collected from the upper 20 cm of the soil profile directly in the root zone at midseason 12–14 weeks after crop establishment in microplots or at 6 weeks in field tests and at harvest. Juveniles were extracted from the samples by elutriation and centrifugation (2) or centrifugal-flotation (7). Eggs were extracted from root residues obtained on a 425-μm-pore sieve during extraction of juveniles by treating the residues with NaOCl (3).

TABLE 2. Population density of *Meloidogyne incognita* eggs and juveniles on flue-cured tobacco in microplots in North Carolina.

Treatment (cultivation and Pi level)	Nematode numbers per 500 cm³ of soil							
	Midseason				At final harvest			
	Eggs	CV	Juveniles	CV	Eggs	CV	Juveniles	CV
Not subsoiled								
0	400*	224	0*		15,100*	130	2,900*	117
750	112,800†	55	2,200	56	117,700	44	43,400†	32
3,000	176,700	29	4,600‡	39	113,300	60	37,200	31
12,000	275,600	46	2,300	62	76,800	64	27,100	34
Subsoiled								
0	0*		0*		8,800*	115	1,400*	101
750	125,200	19	1,800†	8	87,000	28	31,900	50
3,000	135,300	28	4,400	44	72,900	54	30,200	41
12,000	126,800	19	4,200	55	47,300	60	24,800	54

Data are means of five replicates; nematode numbers were transformed to $\log_{10}(X + 1)$ for statistical analyses.
* Statistical differences at $P \leq 0.05$ level of significance for control vs. average of 750, 3,000 and 12,000 Pi levels.
† Statistical differences at $P \leq 0.05$ level of significance for Pi level 750 vs. average of 3,000 and 12,000.
‡ Statistical differences at $P \leq 0.05$ level of significance for Pi level 3,000 vs. 12,000.

TABLE 3. Population levels of *Meloidogyne incognita* and *M. arenaria* based on juveniles and eggs in field experiments in Texas.

Crop and treatment		Nematodes/300 cm³ soil			
		Midseason		At harvest	
		Eggs	Juveniles	Eggs	Juveniles
M. incognita on cotton					
Control		1,060	9	12,600	200
Aldicarb	0.5	60	2	4,300	300
	1.0	80	2	8,700	100
	2.0	80	0	6,800	100
	LSD 0.05	40	4	2,700	NS
M. arenaria on peanuts					
Control		990	70	14,200	300
Aldicarb	1.0	110	37	3,000	20
	0.5 + 1.0	40	20	1,800	20
	LSD 0.05	NS	NS	1,600	NS
M. incognita on cotton					
Control		3,090	70	68,300	800
Aldicarb	1.5	1,790	30	62,000	1,100
	LSD 0.05	500	NS	NS	NS

Differences between numbers of eggs and juveniles were significant at $P = 0.05$ and $P = 0.01$. NS = no significant difference. All data are means of four replicates; data not transformed for statistical analysis. Aldicarb rates are kg a.i./ha; aldicarb was applied as a band treatment with incorporation at planting, except for the split application on peanuts where the second application was an at-pegging treatment.

Data for *Heterodera glycines* Ichinohe were obtained from experiments designed to determine the effects of nematicides, herbicides, and crop rotation systems on population dynamics of this pest. Experiments were established in a deep Wagram fine loamy sand (78% sand, 10% silt, 12% clay, 0.8% organic matter, pH 5.8) in Columbus County, North Carolina, in 1979. A total of 125 plots, each four rows wide (91-cm row spacing) and 12.2 m long, was treated with selected pesticides and planted to soybeans (*Glycine max* (L.) Merr. cv. Davis). Soil samples were taken as outlined for *Meloidogyne* on 29 October 1979 to determine end of season populations. All plots were planted in 1980 to *Zea mays* L. cv. Pioneer 3369A (no nematicides). They were sampled in the spring and in August 1980.

Estimates of Meloidogyne populations: The relative number of eggs obtained in assays was depicted in a nematicide evaluation test involving *Meloidogyne hapla* Chitwood on peanut in microplots in North Carolina (Table 1). Egg numbers, especially at midseason, were considerably greater than the numbers of juveniles. At peanut harvest, numbers of eggs and juveniles were similar in microplot tests. Unfortunately, the common characteristically large variations in nematode population data also occur in measurements of egg numbers as they are deposited in aggregates (egg masses). This fact was reflected by the rather high coefficient of variation (CV) for the numbers of eggs (Table 1).

Midseason numbers of eggs for *M. incognita* on tobacco were as much as 100-fold greater than numbers of juveniles. This differential reflects the importance of monitoring egg populations for early season assays (Table 2). The proportion of eggs also was greater than the proportion of juveniles for final population data. Although counts in this test varied less than those in the previous test, CV were still in the 20–60% range.

Results of the field experiments with *M. incognita* on cotton and *M. arenaria* on peanut were similar to the tobacco test except that the proportion of eggs to juveniles was even greater at harvest (Table 3). Juvenile assays in these tests represented only a small fraction of the nematode population.

TABLE 4. Numbers of *Heterodera glycines* cysts, eggs, and juveniles from 125 soybean (1979) and corn (1980) plots.

Parameter	Soybean		Corn		
	Eggs	Juveniles	Eggs	Cysts	Juveniles
Mean	5,493	170	821	12	8
SD	5,291	161	114	13	16
Min. no.	80	0	0	0	0
Max. no.	25,900	860	7,900	68	68
Positive assays (%)	100	97	83	94	27
Correlation coefficients					
Juveniles	0.45		0.31	0.34	
Cysts			0.62		

Data not transformed for statistical analysis.

Estimates of Heterodera glycines populations: Numbers of eggs following a susceptible soybean crop were greater than numbers of juveniles. In addition, eggs were obtained from all samples of *H. glycines,* and juveniles were detected in 97% (Table 4). Numbers of nematodes following corn provided a more striking comparison of egg and (or) cyst assays with those based on juveniles. As with *Meloidogyne* spp., numbers of eggs were much greater than those of juveniles. More important, the detection rate in samples based on numbers of eggs was 83% and on presence of cysts 94%; however, detection based on juveniles was only 27%. Therefore, egg and cyst assays were much more reliable than those based on numbers of juveniles.

These data illustrate the importance of monitoring numbers of nematode eggs along with other life stages when estimating population densities. Assays of soils suspected to harbor species of *Heterodera* or *Globodera* should include determinations of numbers of eggs and cysts for advisory purposes, particularly following nonhost crops such as corn or during periods (late fall to early spring) when few juveniles would be present. This approach is especially important during periods when the juveniles of these genera are in diapause or an inactive state. Similarly, early season to midseason assays designed to facilitate evaluation of nematicides or potential resistance of cultivars of breeding lines and precise population studies should include numbers of eggs along with numbers of infective stages for *Meloidogyne, Heterodera, Globodera,* and other taxa that produce egg masses or cysts.

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