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Relationship Between Egg Viability and Population Densities of *Meloidogyne incognita* on Cotton

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Abstract: Cotton seedlings were inoculated with a range of initial populations (Pi) of *Meloidogyne incognita* in greenhouse experiments to test the relationship between nematode population densities and egg viability. In two of three experiments, a significant ($P < 0.05$) negative linear relationship was detected between percentage of hatch of first generation eggs and log Pi. A similar relationship between hatch and root-gall index was observed. In two experiments numbers of eggs judged to be nonviable based on appearance were significantly greater ($P < 0.05$) in the highest Pi (60,000 eggs/seedling) treatments than in treatments with lower Pi (600–6,000 eggs/seedling). It was concluded that Pi affects egg viability measured as percentage of hatch and that this relationship may play a role in the density-dependent winter survival rates of *Meloidogyne* species.

Key words: egg hatch, egg viability, *Meloidogyne incognita*, population dynamics, root-knot nematode, winter survival.

Effective nematode management systems utilize information on all aspects of nematode biology, ecology, and interactions with crop species. The dynamics of population survival during relatively long periods of adverse conditions (i.e., winter survival) is one of the areas critical to the development of improved management systems. Population survival during adverse conditions is influenced by many different factors. Some of these factors, such as temperature and moisture, have been studied extensively (10,11). Other factors, such as the influences of the host, intraspecific competition, predators, and pathogens have received relatively little attention.

Both Ferris (3) and Starr and Jeger (6) reported that winter survival of *Meloidogyne* species is inversely density dependent; i.e., populations with a relatively high density in the fall of the year have lower survival rates than do populations at lower densities. Two general hypotheses have been proposed to explain this density-dependent survivorship. One hypothesis states that as nematode populations increase, so do the populations of predators and pathogens, which leads to greater rates of nematode mortality (3). The other hypothesis (3,6) is that increased *Meloidogyne* population densities cause increased competition for the finite resources that can be derived from the host. This results in lower levels of resources (energy reserves) being partitioned into individual eggs; therefore, eggs and resultant juveniles produced at high population densities are less viable or less fit for winter survival than those produced by populations of a lower density. The objective of this study was to determine the effect of nematode population densities on egg viability. A preliminary report has been presented (7).

Materials and Methods

The race 3 population of *Meloidogyne incognita* (Kofoid and White) Chitwood used in these studies was originally isolated from cotton and was maintained on *Lycopersicon esculentum* Mill cv. Rutgers in the greenhouse. Inoculum was collected from 8–10-week-old cultures by the NaOCl method (5).

Single 6-day-old cotton seedlings (*Gossypium hirsutum* L. cv. Tamcot SP37) were planted into 15-cm-d pots containing a washed river sand–peat soil mix (6:1, v/v). A suspension of nematode eggs was placed around the tap root of each seedling at transplanting. Seedlings were maintained in a greenhouse at 24–31 C and fertilized weekly with N-P-K. The experiment was terminated after 6 weeks, and dry shoot weights, root-gall indices, eggs per gram fresh root weight, egg viability, and stage

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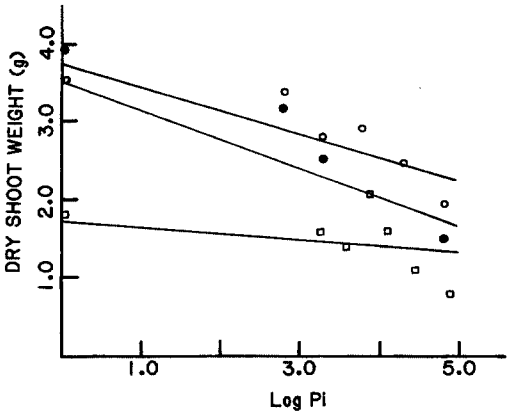

FIG. 1. Relationship between log transformed initial populations (log Pi) of *Meloidogyne incognita* and growth of cotton in three greenhouse experiments. Open circles are experiment I where y = 3.51–0.32 (log Pi), r^2 = 0.73; open squares are experiment II where y = 1.75–0.09 (log Pi), r^2 = 0.38; and closed circles are experiment III where y = 3.75–0.29 (log Pi), r^2 = 0.68. The slope of the regression equation was not significantly different from zero in experiment II.

of egg development were determined. Root-gall index was measured subjectively on a scale of 1 to 6 with 6 equaling greater than 90% root galling (1). The experimental design was a randomized complete block with initial nematode population densities (Pi) as treatments and with 4–6 replications. The experiment was repeated three times with Pi levels ranging from 600 to 60,000 eggs/seedling.

Egg viability was measured as percentage of hatch. Eggs were extracted from the cotton roots using 0.5% NaOCl for a maximum of 4 minutes (5). Aliquots of 200–300 eggs from each plant were placed in hatching chambers constructed from PVC tubing (2.5 cm i.d.) with 20-μm-pore size nylon mesh glued to the bottom. The chambers were placed individually on a 1-mm-pore size support screen in 6.0-mm-d petri dishes containing tap water. Hatched juveniles that migrated through the 20-μm-pore screen were collected every 3 or 4 days for 14 days and counted. In experiments II and III, eggs not used in hatching tests were preserved in 2% formaldehyde. Samples of preserved eggs were subsequently examined microscopically at 100× or 400× to determine distribution of developmental stages within each population. Individual eggs were classified as nonviable, 1 celled, 2–8 celled, 9 celled to blastula, gastrula, J1, or J2. Eggs identified as nonviable had a disorganized appearance with granulated and (or) necrotic contents.

Data from all experiments were subjected to analysis of variance using the SAS general linear models procedure and linear regression analysis (4).

RESULTS

In experiments I and III, dry shoot weight was negatively correlated with log Pi (Fig. 1), whereas eggs per gram of roots and root-gall index were positively correlated with log Pi (Table 1). In experiment II, no effect of *M. incognita* on growth of cotton was observed (Fig. 1), but a significant correlation was observed between log Pi and root-gall index (Table 1).

When eggs collected from each Pi treatment were incubated in hatching chambers for 14 days, percentage of hatch was negatively correlated with log Pi in experiments I and III (Fig. 2). A similar relationship was observed between the root-gall index and percentage of hatch in these ex-

TABLE 1. Relationship between initial populations (Pi) of *Meloidogyne incognita* and cotton dry shoot weight, root-gall index, and eggs per gram of roots in greenhouse tests.

	Number of Pi tested	r^2 of Pi†		
		Dry shoot weight	Gall index	Eggs/g root
Experiment I	4	0.73** (−)	0.85** (+)	0.99** (+)
Experiment II	6	0.38	0.90** (+)	0.37
Experiment III	5	0.69* (−)	0.86** (+)	0.72* (+)

*, ** Indicate significance at P = 0.05 and 0.01, respectively.
† Slopes of regression lines are indicated (−) or (+).

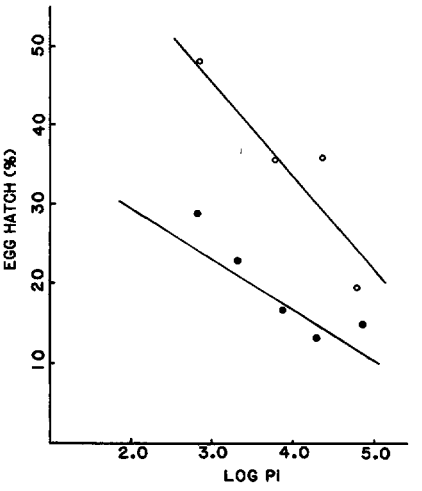

FIG. 2 Relationship between log transformed initial populations (log Pi) of *Meloidogyne incognita* on cotton and percentage of hatch of eggs produced in the first generation. Open circles are experiment I where y = 82.4–12.1 (log Pi), $r^2 = 0.82$; closed circles are experiment III where y = 48.3–7.5 (log Pi), $r^2 = 0.84$.

periments (Fig. 3). In experiment II, however, hatch was not correlated with either log Pi or the root-gall index; hatch for all treatments in this experiment ranged from 24.2 to 31.2%.

No correlation between stage of egg development and either log Pi or root-gall index was detected in experiments I and III. In both experiments, however, the number of nonviable eggs (based on appearance) at the highest Pi was significantly greater than the number of nonviable eggs at the three lowest Pi (Table 2). No significant effect of Pi was noted for the other stages of egg development.

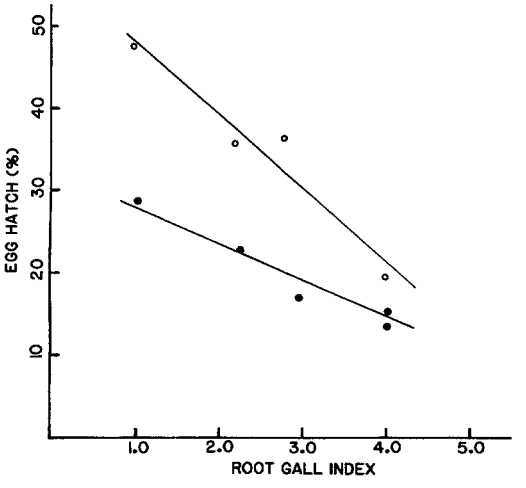

FIG. 3. Relationship between root-gall index of cotton seedlings infected by *Meloidogyne incognita* and percentage of hatch of eggs produced in one generation. Open circles are experiment I where y = 62.2–10.4×, $r^2 = 0.97$; closed circles are experiment III where y = 21.7–1.6×, $r^2 = 0.89$.

TABLE 2. Effect of initial populations (Pi) of *Meloidogyne incognita* on egg development in greenhouse tests.

Pi (eggs/seedling)	Percentage of egg population		
	Nonviable†	Blastula	J2
Experiment II			
1,850	4.6	26.7	14.7
3,700	4.6	24.8	12.9
7,500	3.6	26.2	18.2
15,000	5.2	24.9	14.9
30,000	12.0	24.2	19.8
60,000	9.1	26.3	15.5
LSD 0.05	4.1	NS	NS
Experiment III			
600	4.7	50.0	6.1
2,000	3.8	42.5	7.1
6,000	4.0	37.7	4.3
20,000	7.1	48.2	9.7
60,000	11.5	38.5	5.2
LSD 0.05	3.6	NS	NS

Values are means of six replications of each Pi with a minimum of 50 eggs examined per replication.

† Nonviable eggs were identified based on apparent cellular disorganization, granulation, and (or) discoloration of eggs.

DISCUSSION

The objective of these experiments was to determine if initial population density (Pi) of *M. incognita* affects viability of eggs produced by that population. Two parameters were used to estimate egg viability: percentage of egg hatch, an objective evaluation; and percentage of eggs judged to be nonviable based on appearance, a subjective evaluation. Percentage of hatch in two of three experiments was negatively correlated with Pi and root-gall index. In the experiment where this correlation was not observed (experiment II), Pi did not affect host growth. In experiments II and III where egg viability based on appearance was also assessed, significantly more eggs were judged to be nonviable at the highest Pi than at the lowest Pi. There was

no effect, however, of Pi on percentage egg hatch in one of these experiments (experiment II). This discrepancy between assessment of egg viability based on appearance and that based on percentage of hatch is probably due to the subjective nature of the visual assessment, making it the less reliable method. During visual assessment, data were collected on proportion of eggs in each of eight stages of development. The percentage of eggs in each developmental stage was not affected by Pi. Reduced egg hatch observed at high Pi was not, therefore, due to differences in the rate of embryo development. Thus, the weight of the evidence suggests that as *Meloidogyne* populations increase to high levels resulting eggs have reduced viability.

Egg viability probably declines at high Pi because intraspecific competition results in reduced levels of host resources (energy) being partitioned into the eggs. This is similar to the relationship between Pi of *Meloidogyne* species and the sex ratio of the population. Here also it is assumed that intraspecific competition for host resources triggers a greater percentage of juveniles to develop into males rather than females (9). In the single experiment where no correlation was observed between Pi of *M. incognita* and percentage of hatch, host growth was not affected by Pi. This may represent a case where intraspecific competition was insufficient to affect egg viability.

The data presented herein appear to be contrary to those reported by Storey (8) for a similar study with *Globodera rostochiensis* on potato. He did not detect any effect of Pi on the neutral lipid concentration of unhatched juveniles and concluded that intraspecific competition for host resources did not affect energy reserves of these unhatched juveniles. Storey (8), however, measured the neutral lipid concentration only in eggs containing fully developed juveniles. Portions of the *G. rostochiensis* egg population that did not contain fully developed juveniles and were not examined may have exhibited reduced levels of neutral lipids.

The data presented are consistent with the hypothesis that density-dependent winter survival rates of *M. incognita* are due to effects on egg viability (3,6). This does not in any way negate the alternative hypothesis that as egg and juvenile populations in the soil increase, there will be an accompanying increase in the populations of pathogens and predators. Additional work is needed to test the two hypotheses and to determine relative importance of each in reducing population survival rates at high population densities.

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