

Competition between *Heterodera glycines* and *Meloidogyne incognita* or *Pratylenchus penetrans*: Independent Infection Rate Measurements¹

HADDISH MELAKEBERHAN² AND JYOTIRMOY DEY³

Abstract: Competition on soybean between *Heterodera glycines* (race 3) and *Meloidogyne incognita* or *H. glycines* and *Pratylenchus penetrans* were investigated in greenhouse experiments. Each pair of nematode species was mixed in 3-ml suspensions at ratios of 1,000:0, 750:250, 500:500, 250:750, and 0:1,000 second-stage juveniles or mixed stages for *P. penetrans*. Nematodes from a whole root system were counted and infection rates standardized per 1,000 nematodes (per replication) prior to testing the null hypothesis through a lack-of-fit *F*-test. Although the effect of increasing *H. glycines* proportions on the infection rate of *M. incognita* was generally adverse, the rate deviated significantly from a trend of linear decline at the 75% *H. glycines* level in one of two experiments. All lack-of-fit *F*-tests for the *H. glycines* and *P. penetrans* mix were significant, indicating that infection rates for both nematodes varied considerably across inocula. The infection rate of *H. glycines* decreased with increasing *P. penetrans* proportions. The rate of *P. penetrans* infection increased with increasing *H. glycines* proportions up to the 50% level, but declined at the 75% level. Competition had no effect on nematode development. The general adverse relationships between *M. incognita* and *H. glycines* and those between *P. penetrans* and *H. glycines* showed a linear trend. The relationship between *H. glycines* and *P. penetrans* indicates that the former may be competitive when present at higher proportions than the latter. In this study we have evaluated nematode competition under controlled conditions and provide results that can form a basis for understanding the physical and physiological trends of multiple nematode interactions. Methods critical to data analyses also are outlined.

Key words: Competition, *Glycine max*, *Heterodera glycines*, lesion nematode, *Meloidogyne incognita*, nematode, *Pratylenchus penetrans*, replacement, root-knot nematode, soybean cyst nematode.

The presence of several taxa of plant-parasitic nematodes in agricultural soils is a complication in nematode management (Ibrahim and Lewis, 1986; Niblack et al., 1986; Stetina et al., 1997; Thomas and Clark, 1983). This is particularly challenging for the use of resistant cultivars because most nematode resistance is targeted at one species, which may be present with other nematode species that can parasitize the resistant host (Bradley and Duffy, 1982). For example, *Heterodera glycines* Ichinohe, the most serious pest of soybean, is found in areas where *Meloidogyne incognita* (Kofoid & White) Chitwood and *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven are found (Gay and Bird, 1973; Niblack et al., 1986). From a management perspective, there is a need to understand how the presence of several taxa influences management decisions as well as how the nematodes may affect each other. Plant-parasitic nematodes occupying different niches within a field may parasitize a host without affecting each other; however, nematodes occupying the same niche may affect each other by competing for resources and feeding

sites as well as possibly triggering unfavorable host reactions.

Competition between and among nematodes is a function of initial population density, reproductive potential, host status, and edaphic factors that influence host-parasite interactions. For example, *M. incognita* was suppressed by *Hoplolaimus columbus* in soybeans (Guy and Lewis, 1987) and by *Belonolaimus longicaudatus* in corn (Dickson and McSorley, 1990). The reverse did not happen in either case. However, *P. brachyurus* did not affect *M. incognita* in corn (Dickson and McSorley, 1990). Fluctuations between *M. incognita* and *H. glycines* have been reported on soybean under field conditions (Niblack et al., 1986). To understand the cause-and-effect relationship at the niche level, it is important to quantify how the competing nematodes may replace each other over the duration of their life cycles. However, it is difficult to accurately measure competition between or among nematodes without accounting for the effects of edaphic and other biotic factors on host plant growth which, in turn, may influence nematode reproduction (Burrows, 1987).

Fundamental to unraveling competition and replacement are the events that take place at the nematode-root interface. For example, nematodes with different feeding behaviors may influence each other differently than those with similar feeding behaviors. Establishing competition by feeding behavior can be helpful to understanding host-parasite interactions and may enable us to design management strategies that involve cellular and sub-cellular science.

Received for publication 6 December 2001.

¹ Supported by USDA/NRI Competitive Grants Program contract No. 93-01658.

² Associate Professor of Nematology, Department of Entomology, Michigan State University, East Lansing, MI 48824-1115.

³ Assistant Professor, Integrated Biostatistics Unit, Karmanos Cancer Institute; Departments of Pathology and Urology, Wayne State University, Detroit, MI 48201.

The authors we thank R. S. Hussey and H. R. Boerma of the University of Georgia for the plant material and *Heterodera glycines* and *Meloidogyne incognita* cultures, and G. W. Bird and J. M. Scriber of Michigan State University for critically reviewing the manuscript.

E-mail: melakebe@msu.edu

This paper was edited by Terry L. Niblack.

The objective of this study was to demonstrate how *H. glycines* competes with *M. incognita* or *P. penetrans* (representing neoplastic and destructive feeding behaviors [Dropkin, 1989], respectively) in one generation. The assumption was that if root mass is not affected by factors other than nematodes, infection rates should be stable over the duration of a study; thus, any differences in nematode infection rates when more than one species is present may be attributed to competition.

MATERIALS AND METHODS

Growth conditions: Greenhouse growth conditions were set at 25 ± 2 °C with diurnal cycles of 8 hours dark and 16 hours day with photosynthetically active radiation of 300 to 350 $\mu\text{mol}/\text{m}^2/\text{s}$ at canopy level. Seeds of *H. glycines* susceptible maturity group VI soybean cultivar Tracy M were mass-germinated (Melakeberhan, 1998b) in steam-pasteurized sandy loam soil with 87% sand, 8% silt, and 5% clay; pH 7.0; and 28, 119, 56, 139, and 1,386 kg/ha of nitrate, phosphorus, potassium, magnesium, and calcium, respectively (Melakeberhan, 1998a). One week after germination, 120 seedlings (30 per experiment) were transplanted individually into 15-cm-diam. clay pots containing 800 cm^3 sandy loam soil, labeled, and placed on greenhouse benches in arbitrary order (Melakeberhan, 1998b).

Experiments, nematode treatments, and measurements: In Experiment 1, the effects of increasing proportions of *H. glycines* on the infection rate of *M. incognita*, and vice versa, were assessed. Two days after transplanting, 48-hour-old *H. glycines* and *M. incognita* second-stage juveniles (J2) were applied to soil at ratios of 1,000:0, 750:250, 500:500, 250:750, and 0:1,000, respectively. Each treatment was replicated five times. The required proportions per treatment were pre-mixed to assure equal access to the root system and applied in 3-ml tap water suspensions into 3 to 4 1-cm-diam. holes around the base of the stem (Melakeberhan, 1998b). A set of five control plants was treated with tap water only. The second experiment was a repeat of the first experiment. In Experiment 3, 48-hour-old J2 of *H. glycines* and mixed vermiform stages of *P. penetrans* were applied to soil in the same proportions described for Experiment 1. The fourth experiment was a repeat of the third experiment.

All experiments were terminated 18 days after soil infestation because they were designed to test early competition between nematodes with minimal effect on host growth and to distinguish among the developmental stages of the nematodes. Shoots were dried and weighed, roots were washed free of soil, nodules counted, and fresh root weights determined (Melakeberhan, 1998b). To minimize sampling errors in determining population densities (hereafter referred to as infection rates), the entire root system of each plant was stained and nematodes counted (Melakeberhan,

1998b). For *P. penetrans*, all vermiform stages were counted as one category. *Pratylenchus penetrans* and *H. glycines* can be distinguished easily because of their distinct morphometric characteristics. For the *H. glycines* and *M. incognita* mix, developmental stages were determined as illustrated in Agrios (1997). Because *M. incognita* does not shed the cuticle after molting, it was relatively easy to separate the pre-adult stages of the two nematodes. Third and fourth stages for both nematodes were noted, but grouped together.

Data analysis: Numbers of nematodes and nodules were adjusted to a per-gram-fresh-root basis. Nematode data were analyzed separately for each experiment with regression models to investigate the effect of competition on infection rates. For each nematode in a given inoculum, we divided the number of nematodes per gram root by the proportion of the nematode in the inoculum to compute a standardized infection rate per 1,000 nematodes. The standardized infection rate may be expressed as follows:

$$y' = \frac{y}{1 - x}$$

where y' = standardized infection rate per 1,000 nematodes, y = total number of the nematode per g root, x = proportion of the competing nematode in the inoculum. Note that at the 0:1,000 and 1,000:0 levels the infection rate for only one nematode is estimable.

Lack-of-fit F -tests were employed to test the null hypothesis that varying proportions of a competing nematode in the inoculum has no effect on the infection rate of a target nematode (hypothesis of no effect). If this hypothesis is correct (i.e., if infection of the host by a particular species is independent of the presence and proportion of competing species in the inoculum), its standardized infection rates will be similar in all inocula. Then the standardized infection rates will not differ significantly from a constant rate across all inoculum levels (i.e., there will be no significant deviation from a zero-slope straight line through the overall mean standardized infection rate). Our F -test assesses the lack-of-fit of such a hypothesized line to the observed rates. An F -statistic significant at the 10% level (to account for possibly low statistical power) is considered to indicate that the infection rate of the target nematode was indeed affected by the competing nematode.

RESULTS

Nematode treatments had no effect on nodulation or plant growth (data not shown). About a third of the developmental stages of *H. glycines* and *M. incognita* were third/fourth stages in all experiments. The rest were pre-egg-laying adults. No treatments affected the proportions of nematode developmental stages.

The lack-of-fit F -test showed no deviation from a constant infection rate by *H. glycines* with increasing proportions of *M. incognita* in the inoculum (Table 1; Fig.

TABLE 1. Mean standardized infection rates^a per 1,000 nematodes (\pm SE) of *Meloidogyne incognita* and *Heterodera glycines*.

Experiments	Inoculum ratio					Pvalue ^c
	1000:0 ^b	750:250	500:500	250:750	0:1000	
	<i>H. glycines</i> per g root					
1	6.5 (\pm 0.6)	5.0 (\pm 1.4)	5.5 (\pm 1.8)	6.8 (\pm 2.1)	— ^d	0.36
2	11.7 (\pm 1.3)	10.6 (\pm 1.1)	8.5 (\pm 0.9)	9.1 (\pm 1.5)	—	0.30
	<i>M. incognita</i> per g root					
1	— ^d	7.1 (\pm 0.8)	13.3 (\pm 3.6)	15.3 (\pm 3.0)	19.1 (\pm 3.0)	0.35
2	—	20.5 (\pm 1.6)	14.5 (\pm 1.8)	20.6 (\pm 1.3)	24.3 (\pm 2.2)	0.06

^a Standardized infection rate per 1,000 nematodes were computed as $y/1-x$ where y = total number of the nematode per g root, and x = proportion of the competing nematode in the inoculum.

^b Values represent ratios of *H. glycines* to *M. incognita* per 1,000 total nematodes in the inoculum mixture.

^c Lack-of-fit *F*-test for the effect of increasing proportions of the competing nematode on standardized infection rates.

^d Infection rates estimable for one nematode only.

1A,B). *Meloidogyne incognita* infection rate did not vary with increasing proportions of *H. glycines* in Experiment 1, but deviated from the zero-slope line only at $P \leq 0.06$ level in Experiment 2 (Table 1). Trends were similar in both experiments except that observed *M. incognita* infection rates at the 75% *H. glycines* inoculum level were much higher in Experiment 2 than in Experiment 1, and were also higher than those at the 50% level (Fig. 1C,D).

In Experiments 3 and 4, the lack-of-fit *F*-tests were significant for *P. penetrans* and *H. glycines* (Table 2). *Heterodera glycines* infection rates in both experiments were similar at the 0% and 25%, and at the 50% and 75% *P. penetrans* levels, but dropped off from the 25% to the 50% level (Fig. 2A,B). The infection rate of *P. penetrans* varied non-linearly with increasing proportions of *H. glycines* (Fig. 2C,D). In both experiments, observed rates were similar at the 0% and 25% *H. gly-*

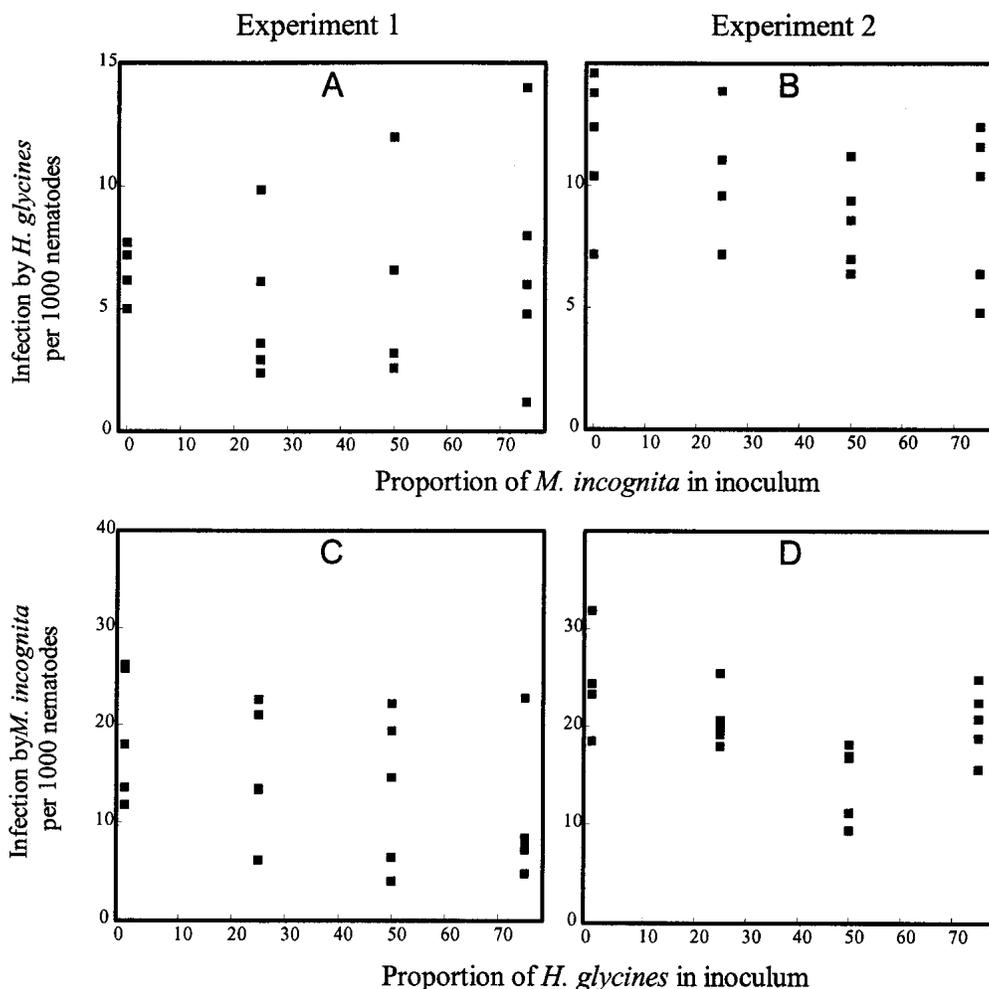


FIG. 1. Scatter plot showing the effect of increasing proportions of *Meloidogyne incognita* on the numbers of *Heterodera glycines* infecting roots of *H. glycines*-susceptible soybean cultivar 'Tracy M' at 18 days after inoculation (A and B) and vice versa (C and D) in Experiments 1 and 2.

TABLE 2. Mean standardized infection rates^a per 1,000 nematodes (\pm SE) of *Heterodera glycines* and *Pratylenchus penetrans*.

	Inoculum ratio					P-value ^c
	1000:0 ^b	750:250	500:500	250:750	0:1000	
	<i>H. glycines</i> per g root					
Experiment 3	18.6 (\pm 5.2)	14.1 (\pm 3.8)	6.2 (\pm 1.6)	5.1 (\pm 1.9)	— ^d	0.06
Experiment 4	18.2 (\pm 1.2)	21.9 (\pm 2.3)	7.8 (\pm 1.7)	13.7 (\pm 2.3)	—	0.01
	<i>P. penetrans</i> per g root					
Experiment 3	— ^d	12.5 (\pm 3.7)	32.4 (\pm 9.2)	22.4 (\pm 2.3)	18.1 (\pm 2.9)	0.02
Experiment 4	—	18.6 (\pm 3.9)	51.0 (\pm 6.3)	28.4 (\pm 3.3)	26.1 (\pm 3.5)	0.01

^a Standardized infection rate per 1,000 nematodes were computed as $y/1 - x$ where y = total number of the nematode per g root, and x = proportion of the competing nematode in the inoculum.

^b Values represent ratios of *H. glycines* to *P. penetrans* per 1,000 total nematodes in the inoculum mixture.

^c Lack-of-fit *F*-test for the effect of increasing proportions of the competing nematode on standardized infection rates.

^d Infection rates estimable for one nematode only.

cines inoculum levels, and were highest and lowest at the 50% and 75% levels, respectively.

DISCUSSION

This study was conducted as part of a project dealing with understanding the physiological basis of multiple

nematode-host interactions for purposes of developing future management options (Melakeberhan, 1997). In studying competition between nematodes, there is a need to (i) separate the effect of the nematodes on each other from several confounding factors and (ii) apply appropriate analyses. When competitions are

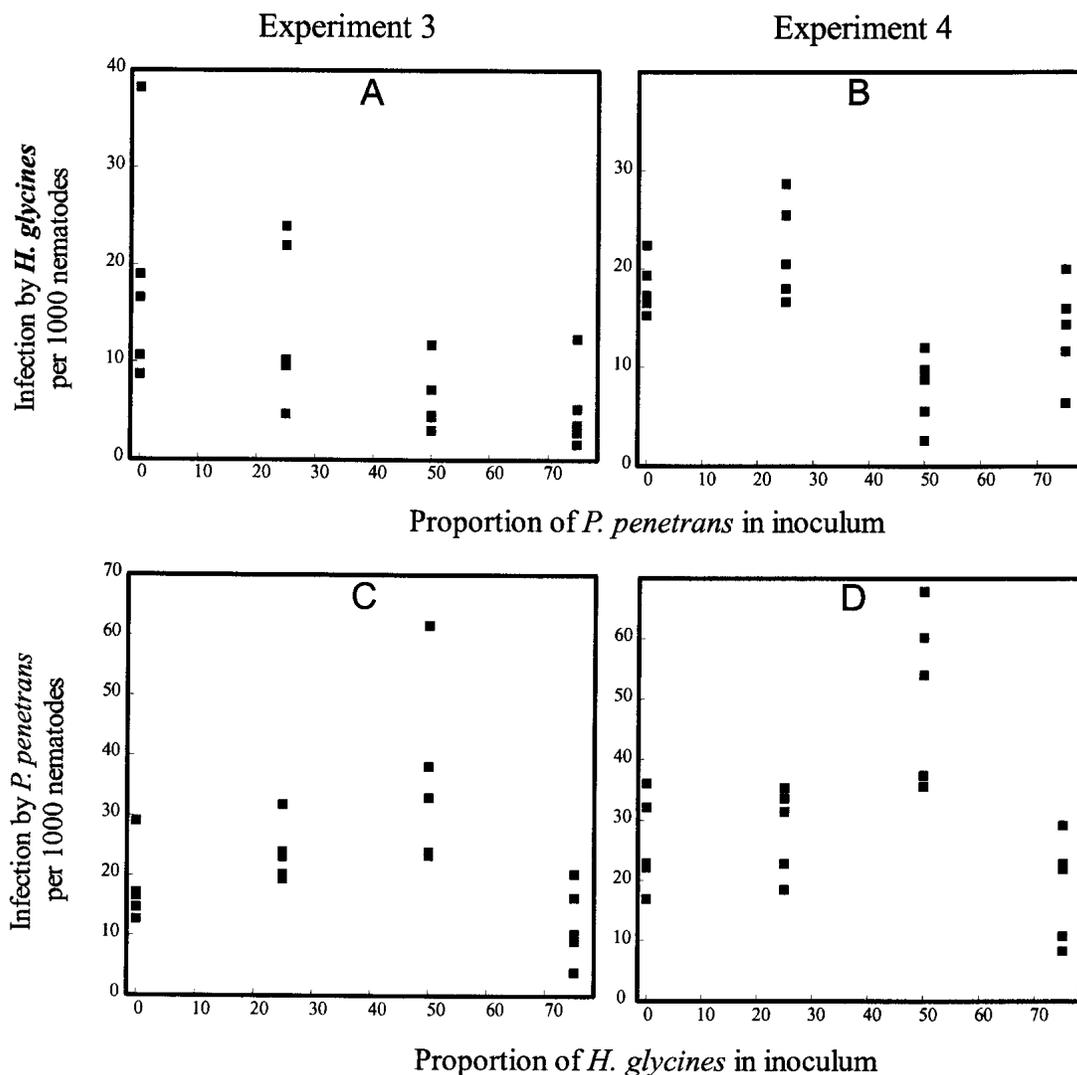


FIG. 2. Scatter plot showing the effect of increasing proportions of *Pratylenchus penetrans* on the numbers of *Heterodera glycines* infecting roots of *H. glycines*-susceptible soybean cultivar 'Tracy M' at 18 days after inoculation (A and B) and vice versa (C and D) in Experiments 3 and 4.

measured over multiple generations, the impact of the nematodes and other stress-inducing factors on host-plant phenology need to be determined simultaneously (Burrows, 1987; Wallace, 1987). This, however, is difficult. Hence, the rationale for examining a single generation of competition was to establish a basis for understanding how nematodes of different behaviors interact with each other with few confounding effects on host growth.

In analyzing data from replacement series experiments, analysis of variance (ANOVA) methods to assess differences in the mean number of nematodes per gram root between inocula are inappropriate for two reasons (Stetina et al., 1997). First, the total number of a specific nematode introduced into soil varies by inoculum. The average number also may differ significantly between inocula even when the rate of infection is not affected by competition. Second, ANOVA does not account for differences in infection and reproduction rates of the target nematode and the competing nematode. This, in turn, leads to disparity in the proportions of the target nematode in the root, at the beginning and at the end of experiments, and from level to level even when it is not affected by competition. Hence, it is necessary to suitably standardize the nematode counts prior to analyses.

In testing the hypothesis of no effect, the manner in which the counts are standardized is very important. For example, Stetina et al. (1997) proposed testing the hypothesis of no effect by standardizing the final count of infecting nematodes in the root system at each level of inoculum. For this, they divided the final counts at each level by the average total count at the no competition (1000:0) level. This assumes that maximum infection takes place in the absence of competition. Thus, if the infection rate of a species in mixed inoculum is not affected by competition, then its standardized final count should be close to its original proportion in the inoculum. A lack-of-fit *F*-test can then be used to detect differences from such a trend and thereby test the hypothesis of no effect. However, in dividing by the same average to compute standardized infection counts, the authors violate an essential assumption for the lack-of-fit *F*-test (i.e., independence of observations).

To avoid these drawbacks, we computed infection rates in different inocula by standardizing the number of nematodes in the root to obtain an infection rate per gram root per 1,000 nematode in each inoculum. The total count per gram root was divided by the proportion of the nematode in the corresponding inoculum. This preserves independence of the rate measurements, allowing use of lack-of-fit *F*-tests, regression, and other common statistical techniques (Neter et al., 1996) for the analysis of replacement series data.

Based on independent rate measurements, our study provides quantitative analyses of the effect of competing nematode species on each other (Tables 1, 2). Fur-

thermore, a combination of the lack-of-fit *F*-tests and inspection of the scatter plots of the data provide better insight into the relationship between the competing nematodes than simply drawing a line through the means. Our results indicate that *M. incognita* and *H. glycines* share a generally adverse relationship. The lack-of-fit *F*-tests were, however, significant only on one occasion. This appears to be due to the high variability of standardized infection rates from one replication to another and possibly low number of replications. Nonetheless, the general decrease in the numbers of either *M. incognita* or *H. glycines* found in root systems with increasing proportions of the other nematode in the inocula shows that both nematodes can be competitive against each other (Table 1; Fig. 1). *Meloidogyne incognita* and *H. glycines* are vermiform until they establish the appropriate feeding sites and become sedentary endoparasites. Hence, the general adverse trends may be related to their similar modes of parasitism and competition for feeding site initiation.

Unlike the *H. glycines* and *M. incognita* mix, the lack-of-fit tests for the former and *P. penetrans* mix were significant, indicating clear competition but varying trends. *Heterodera glycines* infection rates declined almost linearly with increasing *P. penetrans* in the inoculum mix. The increase of *P. penetrans* infection with increasing *H. glycines* up to 50% of the inoculum mix also suggests a linear relationship. It is possible that the migratory feeding behavior of *P. penetrans* may confer an advantage over the sedentary nature of *H. glycines*. However, the sharp decline in *H. glycines* infection rate at 50% and 75% *P. penetrans* in the inoculum, and the decrease in *P. penetrans* infection at 75% *H. glycines* inoculum, suggest some threshold level above which competition may be accelerated (Table 2; Fig. 2).

With research models so variable in duration of study, nematode species, hosts, and experimental conditions, it is not possible to make direct comparisons between studies. Nonetheless, there seems to be a relationship between nematode mode of parasitism and competitiveness. For example, *H. columbus*, a migratory endoparasite, suppressed *M. incognita* in soil nonlinearly, but not the converse (Guy and Lewis, 1987). This seems to support the trends we observed between *P. penetrans* and *H. glycines*. Dickson and McSorley (1990) showed similar trends between *Belonolaimus longicaudatus* (an ectoparasite) and *M. incognita* in corn, but not in the presence of *P. brachyurus*, a migratory endoparasite. Either fluctuations in infection rates (Dickson and McSorley, 1990) or similar inverse relationships (Niblack et al., 1986) between *M. incognita* and *H. glycines* have been reported under field conditions.

How the nature of competition observed here may fluctuate over multiple generations is unknown. A significant effect on the infection rates, but not on the developmental stages, indicates that once a nematode is in the root it can grow and compete. Establishing a

feeding site is one of the critical factors in nematode reproduction and competition. It is likely that the physiological and biochemical changes competing nematodes induce at the feeding sites may trigger host response(s) which, in turn, may hold keys to unraveling the mechanisms of host-parasite interactions. Establishing the trends of competition under controlled conditions will provide a basis for understanding the physical and physiological processes by which the nematodes compete against each other.

LITERATURE CITED

- Agrios G. 1997. *Plant Pathology*. New York: Academic Press.
- Bradley, E. B., and M. Duffy. 1982. The value of plant resistance to soybean cyst nematode: A case study of 'Forrest' soybeans. USDA/ERS Publication No. AGES820929.
- Burrows, P. M. 1987. Interaction concepts for analysis of responses to mixtures of nematode populations. Pp. 82-93 in J. E. Veech and D. W. Dickson, eds. *Vistas on nematology*. DeLeon Springs FL: E. O. Painter Printing Co.
- Dickson, D. W., and R. McSorley. 1990. Interaction of three plant-parasitic nematodes in corn and soybean. Supplement to the *Journal of Nematology* 22:783-791.
- Dropkin, V. H. 1989. *Introduction to plant nematology*. New York: John Wiley & Sons, Inc.
- Gay, C. M., and G. W. Bird. 1973. Influence of concomitant *Pratylenchus brachyurus* and *Meloidogyne* spp. on root penetration and population dynamics. *Journal of Nematology* 5:212-217.
- Guy, D. W., and S. A. Lewis. 1987. Interaction between *Meloidogyne incognita* and *Hoplolaimus columbus* on Davis soybean. *Journal of Nematology* 19:346-351.
- Ibrahim, I. K. A., and S. A. Lewis. 1986. Interrelationships of *Meloidogyne arenaria* and *M. incognita* on tolerant soybean. *Journal of Nematology* 18:106-111.
- Melakeberhan, H. 1997. Role of plant nutrition on alleviating nematode parasitism. Pp. 759-760 in T. Ando, K. Fujita, T. Mae, H. Matsumoto, S. Mori, and J. Sekiya, eds. *Plant Nutrition for Sustainable Food Production and Environment*. London: Kluwer Academic Publishers.
- Melakeberhan, H. 1998a. Effects of temperature and nitrogen source on tomato genotypes response to *Meloidogyne incognita* infection. *Fundamental and Applied Nematology* 21:25-32.
- Melakeberhan, H. 1998b. Pathogenicity of *Pratylenchus penetrans*, *Heterodera glycines*, and *Meloidogyne incognita* on soybean genotypes. *Journal of Nematology* 30:93-98.
- Neter J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. *Applied Linear Statistical Models*, 4th ed. New York: McGraw-Hill Book Co.
- Niblack, T. L., R. S. Hussey, and H. R. Boerema. 1986. Effects of interactions among *Heterodera glycines*, *Meloidogyne incognita*, and host genotypes on soybean yield and nematode population densities. *Journal of Nematology* 18:436-443.
- Stetina, S. R., J. S. Russin, and E. C. McGrawley. 1997. Replacement series: A tool for characterizing competition between phytopathogenic nematodes. *Journal of Nematology* 29:35-42.
- Thomas, R. J., and C. A. Clark. 1983. Effect of concomitant development on reproduction of *Meloidogyne incognita* and *Rotylenchus reniformis* on sweet potato. *Journal of Nematology* 15:215-221.
- Wallace, H. R. 1987. Effect of nematode parasites on photosynthesis. Pp. 253-259 in J. E. Veech and D. W. Dickson, eds. *Vistas on nematology*. DeLeon Springs FL: E. O. Painter Printing Co.