Relationships of Initial Population Densities of *Meloidogyne incognita* and *M. hapla* to Yield of Tomato[1]

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Abstract: Microplots 80 x 100 cm, infested with varying initial population densities (P_i) of *Meloidogyne incognita* or *M. hapla*, were planted to tomato at two locations. Experiments were conducted in a sandy loam soil at Fletcher, N. C. (mountains) where the mean temperature for May to September is ca 20.7 C, and in a loamy sand at Clayton, N. C. (coastal plain) where the mean temperature for May to September is ca 24.8 C. In these experimentally infested plots, *M. incognita* and *M. hapla* caused maximum yield losses of 20-30% at the mountain site with P_i of 0-12,500 eggs and larvae/500 cm^3 of soil. In the coastal plain, *M. incognita* suppressed yields up to 85%, and *M. hapla* suppressed yields up to 50% in comparison with the non-infested control. A part of the high losses at this site apparently was due to *M. incognita* predisposing tomato to the early blight fungus. In a second experiment, in which a nematicide was used to obtain a range of P_is (with P_i as high as 25,000/50 cm^3 of soil) at Fletcher, losses due to *M. incognita* were as great as 50%, but similar densities of *M. hapla* suppressed yields by only 10-25%. Approximate threshold densities for both species ranged from 500 to 1,000 larvae and eggs (higher for surviving larvae) for the mountain site, whereas numbers as low as 20 larvae/500 cm^3 of soil of either species caused significant damage in the coastal plain. Chemical soil treatments proved useful in obtaining various initial population densities; however, problems were encountered in measuring effective inoculum after such treatments, especially in the heavier soil. *Key Words:* root-knot nematode, population dynamics, control.

Several experimental approaches have been employed to determine relationships between initial nematode population densities and crop yields. These approaches have included: the use of microplots (7, 8), the utilization of various nematicides under field conditions (11, 14), and the use of greenhouse experiments (15). In attempting to relate initial numbers of *Meloidogyne javanica* (Treub) Chitwood and *M. hapla* Chitwood to the growth of tomato under greenhouse conditions, Swarup and Sharma (15) found that rather high numbers of nematodes were needed to suppress growth significantly. However, numbers as low as 100 larvae/400 gm of soil caused some stunting. Considerable progress has been made in attempting to characterize host responses to nematodes and the development of mathematical models depicting these relationships (14). However, additional data are needed to relate the effects of different nematode densities to yields for specific nematode-crop associations at different locations. Such information is prerequisite to development of nematode assay and advisory programs (2).

The primary objective of this investigation was to determine the relationships of different initial densities of *Meloidogyne incognita* (Kofoid & White) Chitwood and

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M. hapla, respectively, to total and marketable yields of tomato under different soil and climatic conditions as they occur at Clayton, N. C. (coastal plain—elevation of 100 m) and at Fletcher, N. C. (mountains—elevation of 631 m). Secondary objectives of this investigation concerned a study of the population dynamics of these nematode species in different environments and an evaluation of the usefulness of nematicides in establishing different population densities for determining quantitative nematode-plant relationships. Observations were also made on the effects of nematode densities on the predisposition of tomato to *Alternaria solani* at each location. A preliminary report has been published (1).

MATERIALS AND METHODS

Preparation of inoculum and infestation of microplots: Meloidogyne hapla and *M. incognita* were increased individually on tomato, *Lycopersicum esculentum* Mill. 'Floradel' in the greenhouse. Plants were grown for 3 months in infested soil consisting of a 1:1 mixture of loamy sand and a silica sand with maximum diam of 245 μm in 15-cm-deep flats. Nutrients were provided as needed. Inocula for the microplots were prepared by chopping the infected roots into small fragments and incorporating them into the infested soil from the flats used for increasing the inocula. Inoculum used in the first year was increased by inoculating six tomato seedlings in these flats with 30 egg masses/plant. Noninfected plants were also grown in the same fashion for use in diluting the inoculum and for use in the controls.

Microplots, 80 x 100 cm, were made with fiberglass barriers extending 50 cm below and 10 cm above the soil surface. At Clayton, these barriers were installed in an Appling loamy sand (91% sand, 3.3% clay, and 5.7% silt) with a minimum disturbance of the soil profile. A backhoe was used at Fletcher to excavate areas for barrier placement in heavy clay soil. After 5-cm layers of 1 to 2-cm gravel were placed in the bottoms of each plot at the latter location, the plots were filled with a sandy loam soil (73.2% sand, 6.7% clay, 20.1% silt). Before the nematode inoculum was added, all plots were fumigated with methyl bromide (1.5-2.0 Kg/9m^2). Varying amounts of infested and noninfested soil were incorporated in the plots to give the desired range of initial densities for a given nematode species.

Nematode assay procedures: Soil samples for nematode assays were obtained by collecting 10 cores with a 2.5-cm diam soil tube inserted to a depth of 20 cm in each plot. Initial samples were collected ca 1 week after infestation. Subsequent sampling was done monthly or at midseason and at harvest. After composite samples were thoroughly mixed, 50 cm^3 of each sample were used for larval extractions, and 250-500 cm^3 were used for determination of eggs. Larvae generally were extracted by the sugar-flotation-sieving method (4). Where nematodes were extracted within 4 weeks after chemical soil treatments, the Baermann funnel was utilized. Numbers of eggs were determined by the procedure recently developed by Byrd et al. (3).

Experimental designs and culture of plants: Two types of experiments were done at each location. In the initial tests at Fletcher, a range of initial densities of *Meloidogyne incognita* (1972, 1975) and *hapla* (1972) were established by adding the desired numbers of eggs and larvae. This type of experiment was limited to *M. incognita* at Clayton (1971, 1975). The second type of experiment involved the use of the nematicide, fensulfothion (Dasanit® 15-G), which was applied at different rates to re-establish a range of effective initial densities (P_{ie}) of nematodes. For these experiments, the initial numbers of larvae were determined prior to treatment and 3 to 4 weeks later.

In a third type of experiment at Clayton, tomato was transplanted into microplots in which soybean had been the previous crop (1973). Twelve plots (plus 4 noninfested control plots) were infested with *M. hapla* and the same number of plots with *M. incognita.* No additional nematodes were added to these plots.

A randomized, complete block design for each nematode species was used in all experiments. In the initial experiments at both locations, four artificially established, initial-density ranges of nematodes were used in each case. These included: four plots with no nematodes, four plots with low numbers of nematodes and eggs (200-800/500 cm^3 of soil), four with a moderate

TABLE 1. Rainfall and temperature at experimental sites.

Parameter	Month[a]			
	May	June	July	August
Rainfall (Mean-cm)				
Clayton	8.1	7.9	17.4	11.0
Fletcher	20.9	14.9	16.4	16.2
Temperature (Mean low and high (C)				
Clayton				
Low	12.2	16.3	22.1	18.4
High	27.0	30.5	31.1	29.4
Fletcher				
Low	9.0	13.5	16,2	16.2
High	22.9	26.7	28.4	28.1

[a]Data based on 1971 and 1972 for Clayton and 1972 and 1973 for Fletcher.

number of larvae and eggs (1,000-2,200/500 cm³ of soil), and four with a high number of larvae and eggs (4,000-8,000/500 cm³ of soil). For the experiments with the nematicide, four treatments (rates of chemical) plus noninfested (no nematodes) plots were used with three replicates/treatment. Four, 5-week-old 'Manapal' tomato seedlings were transplanted to each plot in mid-May to early June. Standard practices for growing trellised tomatoes were followed. During periods of low rainfall at Clayton, microplots were irrigated minimally with ca 1.7 cm of water/week. Mean monthly rainfall and air temperatures for each test site are summarized in Table 1. At weekly harvests, fruit was graded on the basis of marketable and total yields for each plot. Plant growth was rated at mid- and late season, and gall indices were recorded at the end of the season for most experiments.

For analysis of variance, yield data were grouped according to the four P_i ranges: none (0), low (200-800), moderate (1,000-2,000), and high (4,000-8,000). Results of these analyses were a factor in estimating economic threshold ranges.

Regression analyses also were used to relate total and marketable yields (dependent variable) to initial numbers of nematodes (independent variable) in each experiment. For these analyses, the numbers of nematodes were transformed to $\log_{10}(X + 1)$. Polynomial models were fitted, and the choice of specific degree for each situation was based upon tests of significance for the various power terms. In most cases, the choice was between linear and quadratic models. Tests for parallel slopes were carried out for both locations and years. Although most experiments were repeated in different years, data for only 1 representative year for each type of test are presented herein.

RESULTS

Yields in microplots infested with various densities: The influence of similar initial densities of *Meloidogyne* spp. on the yield of tomato differed greatly at the two sites. The lowest experimentally established initial density (P_i) of *M. incognita* used at Clayton (200/500 cm³) caused severe losses (Table 2, Fig. 1-A, C). At Fletcher, however, similar densities of this species caused only slight losses in total or marketable yields (Table 2, Fig. 1-E). A P_i of 1,000 or more of *M. hapla* was required to cause a significant yield loss at Fletcher. A very low P_i (20/500 cm³ soil) of this species (Fig. 1-B), as well as of *M. incognita* (data not included), caused moderate losses following soybean at Clayton. The growth indices, especially in late midseason, were closely related to yields and are not presented herein.

The relationship of $\log_{10}$ of P_i to total or marketable yield was adequately described by linear regressions for either

TABLE 2. Tomato yields as influenced by initial inoculum densities (P_i) of *Meloidogyne* species.

P_i[a]	Total yield (Kg)[b]		
	Clayton (1971)	Fletcher (1972)	
	M. incognita	*M. hapla*	*M. incognita*
None (0)	17.2	18.2	15.6
Low (200-800)	7.0**	17.5	13.5*
Moderate (1,000-2,200)	5.2**	14.6*	12.3**
High (4,000-8,000)	3.0**	13.5*	12.4**
LSD:			
$P=0.05$	1.4	3.0	1.7
$P=0.01$	2.0	NS	2.5

[a]Range of artificially established eggs and larvae/500 cm³ of soil (specific densities plotted in Fig. 1).
[b]Asterisks (* and **) indicate significant difference ($P=0.05$ and 0.01 respectively) as compared to noninfested (0) control.

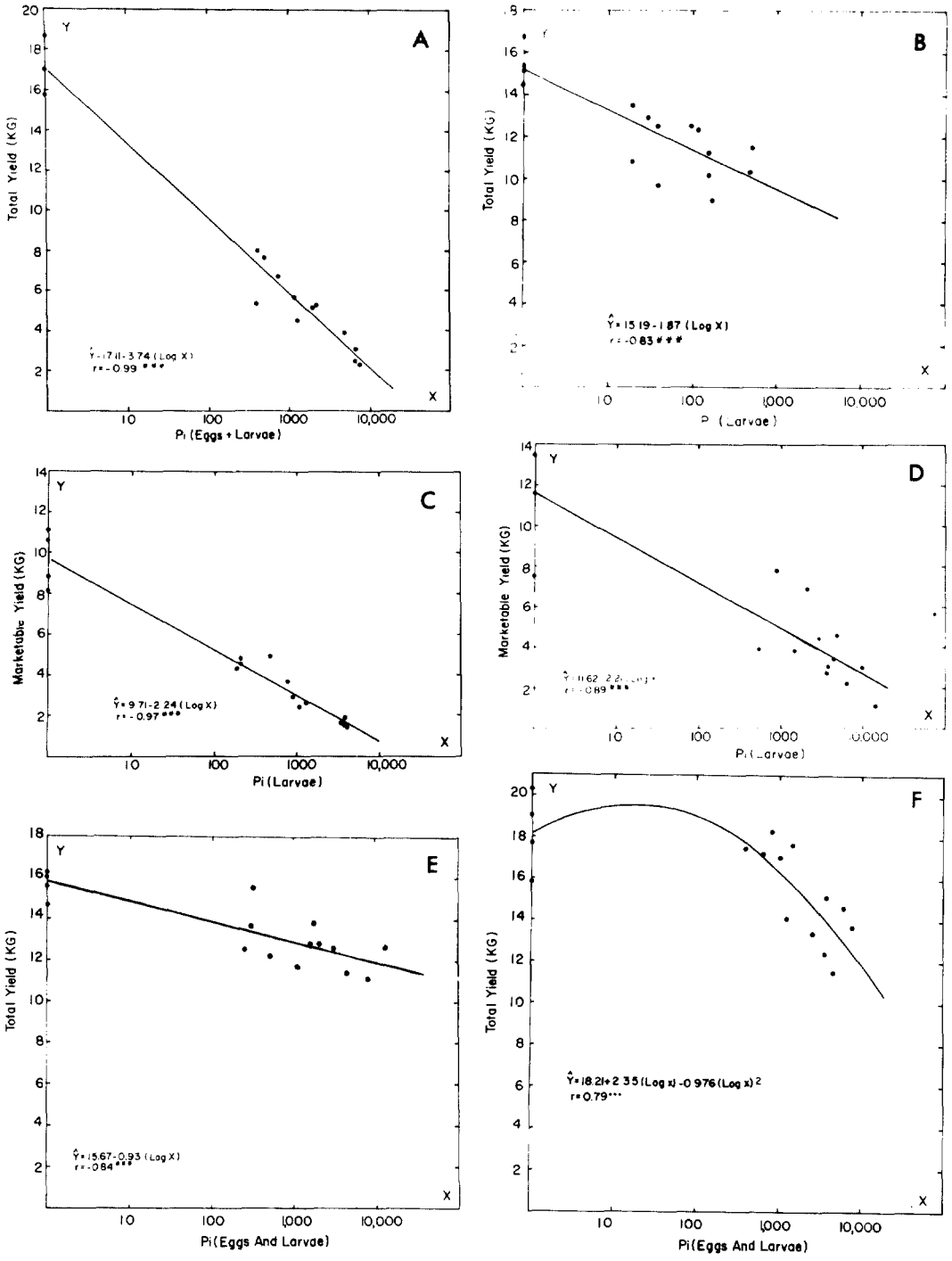

FIG. 1. (A-F). Regressions of *Meloidogyne* spp. to yield of tomato in the coastal plain (Clayton—1971-1973) and mountains (Fletcher—1972) of North Carolina. *A) Meloidogyne incognita* at Clayton (after initial infestation). *B) Meloidogyne hapla* at Clayton (after soybean). *C & D) M. incognita* for year 1 (plots infested with varying P_is) and year 2 (fensulfothion used at varying rates to establish different densities), respectively at Clayton. *E) M. incognita* at Fletcher. *F) M. hapla* at Fletcher (Log X = $\log_{10} P_i + 1$).

species at Clayton (Fig. 1 A-D). At Fletcher, however, the relationship for *M. hapla* and yield was not described as well by a linear relationship but was characterized by a quadratic model (Fig. 1-F). The slopes for *M. incognita* at Clayton (−2.60 to −3.95 log X) were much steeper than those for the nematode at Fletcher (−0.67 to 1.22).

Final nematode population (P_f) differed with location (Fig. 2). For the first experiments, P_f of *M. incognita* declined with increasing P_i at Clayton, whereas it increased with increasing P_i at Fletcher, except for the highest P_i. The P_f for *M. hapla* likewise increased with increasing P_i at Fletcher. Root-knot severity indices were highly correlated with nematode numbers (P_f) and are not included herein.

Use of a nematicide in obtaining a range of initial densities: Chemical soil treatment with fensulfothion was more effective in establishing a range of nematode densities at Clayton than at Fletcher (Table 3). Only the highest rate, however, prevented build-up at Clayton during the season. At Fletcher, nematode populations were not appreciably reduced until midseason, and build-up occurred again by fall.

Total yield in *M. incognita*-infested plots was highly correlated with early-season nematode counts (r = −0.93**) at Clayton but poorly correlated at Fletcher (r = −0.57*). Compared to the noninfested plots, a P_{ie} (count 3-4 weeks after treatment) of 1,200/500 cm³ of soil resulted in a severe yield loss at Clayton, whereas a much higher P_{ie} (9,000-18,000) caused only a moderate loss at Fletcher. Losses due to *M. hapla* infestations as high as 16,800 larvae/500 cm³ were slight (<25%), regardless of chemical soil treatment (Table 3).

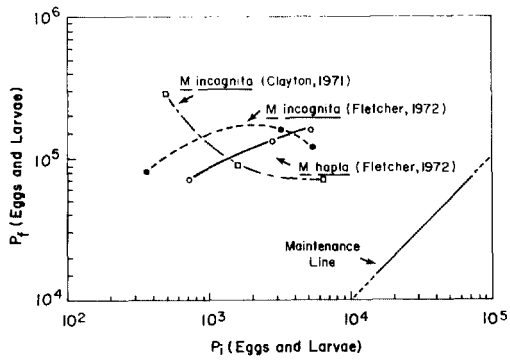

FIG. 2. Relationship of initial population densities (P_i) to final densities (P_f) of *Meloidogyne* spp. in coastal plain (Clayton) and mountains (Fletcher) of North Carolina.

Correlation of early blight development with initial nematode densities and yield: Since early blight *(Alternaria solani)* developed in some plots about 2 months before the final harvest, blight indices and numbers of lesions on the top five leaves/plant were recorded. At Clayton (coastal plain) in 1971, the numbers of lesions were positively correlated with initial numbers (P_i) of *M. incognita* [$\hat{y} = 1.8 + 22.6$ ($\log_{10}$ P_i); r = 0.75**]. Total and marketable yields were negatively correlated with blight development in this experiment [with marketable yield for example, $\hat{y} = 11.33 - 4.66$ ($\log_{10}$ No. lesions); r = −0.88***]. The incidence of this disease was more restricted in subsequent experiments at Clayton. Only limited early blight developed in any experiment in the mountains, and there was no apparent relationship to nematode densities.

DISCUSSION

The results with artificially infested plots and with the nematicide fensulfothion support the hypothesis that economic threshold densities for *M. incognita* are lower at Clayton (coastal plain) than at Fletcher (mountains). Striking losses from nematodes in the coastal plain were not paralled by losses in the mountains, as one might expect. The major differences in yield at the two locations are probably due to climatic differences (temperature, rainfall) and soil type. The greater incidence of early blight at Clayton in some years, compared to the incidence of that at Fletcher, apparently was partly responsible for a corresponding decrease in yield. Results at Fletcher indicate that *M. incognita* is more destructive than *M. hapla* on tomato in the mountains. Differences in the slopes at the two locations (when the controls were included in the linear regressions) were partially due to the higher threshold densities for both nematode species at Fletcher. If the data for the noninfected controls were omitted from the regression analyses, the slopes of all curves/nematode species were similar. Experiments at Fletcher with greater numbers of inoculum levels and replicates may have given

TABLE 3. Relationship of nematicide-altered populations of *Meloidogyne* spp. to yield of tomato at two locations.

Parameter [Location, nema spp. and range of fensulfothion/plot (grams)]	Larval densities/500 cm³ of soil (in 1,000's)[a]			Total yield (Kg/plot)
	P_{ie}	P_m	P_f	
Clayton, N. C. (coastal plain—1972)				
Meloidogyne incognita				
None	9.0	91.0	5.0	2.9
Low (0.8-1.5)	4.0	42.5	9.9	4.8
Moderate (3.0-5.0)	3.1	3.3	23.8	6.9
High (10-15)	1.2	2.1	1.1	9.4
Noninfested plots	0	0	0	20.8
LSD: $P=0.05$	2.5	41.1	NS	2.5
$P=0.01$	3.8	62.3		3.7
r(total yield vs. P) =	— 0.93***	— 0.95***	— 0.53*	
Fletcher, N. C. (mountains—1973)				
Meloidogyne incognita				
None	18.2	134.4	41.6	8.4
Low (0.8-1.5)	20.3	14.5	61.4	12.5
Moderate (3.0-5.0)[b]	9.0	9.4	33.8	16.0
High (10-15)	18.6	10.0	38.7	15.2
Noninfested plots	0	0	0	16.7
LSD: $P=0.05$	NS	NS	NS	5.2
r(total yield vs. P) =	— 0.57*	— 0.65**	NS	
Meloidogyne hapla				
None	16.8	21.7	21.6	13.1
Low	16.4	9.5	26.9	15.0
Moderate	12.7	1.7	7.9	14.6
High	13.0	3.4	16.5	15.2
Noninfested plots	0	0	0	17.1
LSD: $P=0.05$	NS	NS	NS	NS
r(total yield vs. P) =	— 0.59*	— 0.56*	— 0.63**	

[a] P_{ie} = estimated effective initial density, 3-4 weeks after treatment (number larvae recovered by Baermann funnel); P_m = midseason density; P_f = final density. Asterisks (*, **, and ***) indicate significant correlation between yield and nematode density ($P=0.05$, 0.01, and 0.001, respectively).
[b] Mean initial density for plots receiving this treatment was lower than other *M. incognita*-infested plots at Fletcher.

sigmoid curves for P_i vs. yield as described by Seinhorst (12, 13).

Threshold densities or regression equations obtained in one geographic location are not transferable to another geographic situation. Variation in results from year to year also increases the difficulties in predicting effects of nematodes on yield. Another problem encountered in describing plant responses to nematodes involved the transformation of nematode population data. As indicated by Wallace (16), the same population data plotted on a logarithmic scale and an arithmetic scale give very different curves. Analyses of nontransformed data gave highly significant quadratic as well as linear regressions. Since estimates of threshold densities may be influenced by the type of transformation and plotting employed, results of analysis of variance should also be utilized in the development of these constants.

The use of nematicides in establishing various population densities gave useful results in some cases, but this approach also has limitations. During the second year at Clayton when fensulfothion was used at varying rates, *M. incognita* was almost eradicated in plots receiving the highest rates. This response gave very high correlations between surviving larvae and crop yields, but the nematode population dynamics were abnormal. Although we encountered considerable numbers of nematodes in the early season after fensulfothion was used, by the end of the season there were very few nematodes in plots which received the high rates. Such responses alter

the usual relationship between the measurable, initial population numbers of nematodes and the final population, i.e. the reproductive rate (6, 9). Certain nonfumigant nematicides have been shown to alter nematode behavior without inducing death (6). Fensulfothion applied in the mountains on *M. incognita* and *M. hapla* failed to give early-season kill of either nematode species, perhaps because of the effect of soil type on efficacy of this chemical. The reduced nematode activity, however, resulted in considerable yield increase in plots infested with *M. incognita*. Thus, the very different responses of *M. incognita*, as well as crop responses at the two locations to the same nematicide, indicate that prediction of expected returns as suggested by Seinhorst (14) cannot be done without considering effects of soil type, temperature, and rainfall which may vary with location.

The information obtained in these studies should prove useful in evaluating the effects of varying numbers of nematodes on expected yields of tomato at two distinct geographic locations in North Carolina. In the coastal plain (sandy soils and warm climates), almost any number of root-knot nematodes would warrant the use of a nematode-resistant variety and/or a chemical soil treatment for this crop. However, in the mountains (sandy loam soil and relatively cool climate), the use of a chemical soil treatment would be justified with only moderate to high numbers (>500-1,000/500 cm^3 of soil) unless other interacting root pathogens are present. The population (and threshold densities) estimates for both species in soil that had previously supported soybean at Clayton were probably low because of the lack of a satisfactory method for recovering "free" eggs in the soil. With experiments which involve methyl bromide-treated soil, the low numbers of nematodes may have caused less damage than normal, possibly because of the absence of many fungi and bacteria that often occur in field situations. Powell (10) has found that, on tobacco, these nematodes often predispose the roots to attack by a number of pathogenic fungi as well as by normally saprophytic fungi.

More extensive studies of interactions of varying densities of nematodes with certain foliage and root pathogens are needed to give a better understanding of the direct and indirect roles of each organism in the development of disease under field conditions. Although the results for the first 2-4 years at a given site were quite comparable, the damage caused by nematodes may vary from year to year. Under ideal growing conditions, nematodes may cause only moderate damage, whereas under periods of drought or other stress factors, they may cause considerably more damage (16). Therefore, these data can be used only as general guidelines and cannot be depended upon to offer precise predictions of crop losses on the basis of initial infestations.

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