Yield Response and Injury Levels of *Meloidogyne incognita* and *M. javanica* on the Susceptible Tobacco 'McNair 944'[1]

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Abstract: The effects of *Meloidogyne incognita* and *M. javanica* on a susceptible tobacco (*Nicotiana tabacum* L.) cv. McNair 944 were investigated in field microplots during 1978 and 1979. Three initial inoculum levels—4, 16, and 64 nematode eggs and/or second-stage larvae per 100 cm³ of soil—were used for each nematode species. Data obtained from the experiments included plant yield and the amount of reproduction of the two nematode species. At comparative inoculum levels, *M. javanica* was more aggressive than *M. incognita* on tobacco and caused approximately twofold more yield suppression than *M. incognita*. The calculated initial population of *M. incognita*, derived from the average for 2 yr, which produced a 7% suppression in plant yield was four eggs and/or second-stage larvae per 100 cm³ of soil; whereas less than one *M. javanica* egg and/or second-stage larvae per 100 cm³ of soil was needed to achieve similar suppression. Nematode reproduction varied in the 1978 and 1979 tests, but similar trends were observed. Early season *M. javanica* populations were greater than those of *M. incognita*, but late season populations of *M. incognita* were twice and three times those of *M. javanica*. *Key words:* aggressiveness, injury levels, *Nicotiana tabacum*.

Studies have shown the importance of two root-knot nematode species, *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub.) Chitwood, in Florida tobacco (*Nicotiana tabacum* L.) production (18,21). Of these two species, *M. incognita* is considered the most widespread but *M. javanica* causes the most damage in Florida tobacco. These observations are consistent with studies of Barker (3) who found that four root-knot nematode species differ in their ability to reduce tobacco yield. His regression model analysis indicated yield losses from a susceptible tobacco would be 19.9, 16.5, 8.8, and 3.7% for *M. javanica, M. arenaria, M. incognita,* and *M. hapla,* respectively, for each 10-fold increase in initial inoculum density.

A common approach to determine injury levels and nematode aggressiveness has been to study the relationship between nematode population levels and crop yield. Methods to accomplish this have included the use of microplots (4,15,20), the utilization of various nematicides under field conditions (6,10,19), and the use of greenhouse and growth chamber experiments (7,11).

In Florida only empirical evaluations of nematicide experiments have been used to establish injury levels of root-knot nematodes on tobacco. Because of our need to improve predictive nematode management techniques, this study was conducted to establish the injury levels of *M. incognita* (Race 1) and *M. javanica* on tobacco and to determine if one species was more aggressive than the other under Florida conditions.

Aggressiveness in the context of this study is defined as the relative degree to which a pathogenic potential is expressed. Injury level is defined as the nematode density which produces a specified level of plant damage.

MATERIALS AND METHODS

In the fall of 1977 a Lakeland fine sand soil (93.1% sand, 3.9% silt, and 3.0% clay) was treated with 988.8 kg/ha methyl bromide; 2 months later 64 fiberglass cylinders (76-cm-d) were installed in the soil (20). The microplot area was fallowed over winter until planting. Before planting the microplots were sampled and no plant-parasitic nematodes were recovered.

Eggs and second-stage larvae for inoculum were extracted from roots of tomato (*Lycopersicon esculentum* Mill.) cv. Rutgers with 1% sodium hypochlorite (12). On 6 April 1978 microplot soil was infested with 4, 16, and 64 *M. javanica* or *M. incognita* eggs and/or second-stage larvae per 100 cm³ of soil by adding 20-ml of an aqueous nematode suspension at the appropriate concentration to each of 55 evenly spaced holes (8, 15, and 23 cm deep) within each microplot. One day later two plants of the root-knot

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nematode susceptible tobacco cv. McNair 944 were planted 25 cm apart in each microplot. Eight replicates per treatment were arranged in a randomized complete block design.

Recommended fertilization rates under field conditions were used. The microplots were kept weed free, irrigated, and sprayed for insects as needed to promote good plant growth and development. Sixty-four days after planting, and as needed thereafter, axillary and terminal buds were removed. No sucker control agents were applied.

Sixty and 124 d after inoculation, five soil cores (2.84 × 30 cm deep) were taken from each microplot and mixed; 250 cm^3 of soil was then processed by a modified centrifugation-sugar-flotation technique (13). The soil samples were taken 10 cm from the plant crown, and sampling sites were different for each sampling date. Tobacco roots from a 250-cm^3 aliquant of soil from each microplot were placed on Baermann funnels for 3 d (2). The number of nematodes inside the roots was determined. The total number of root-knot nematodes recovered from soil and roots was converted to nematodes per 100 cm^3 of soil. After the last harvest, 124 d after planting, the tobacco roots systems were rated for galling according to the following index: 0 = no galls, 1 = trace of galling (1–25%), 2 = moderate galling (26–50%), 3 = severe galling (51–75%), and 4 = very severe galling (76–100%) (16).

Mature tobacco leaves were harvested five times: 70 d after planting and every 10 d thereafter. The leaves were dried at 49 C before being weighed. Average soil temperatures at a 10.2-cm depth and precipitation during April, May, June, and July were determined.

A similar experiment was conducted in 1979. Inoculum levels and inoculation procedure was as described above. Four soil cores were taken in each microplot 30, 60, 90, and 104 d after inoculation. Handling of soil, nematode population determination, root-gall rating, and tobacco leaf harvest were as in 1978. Thirty days after planting, tobacco roots from one 250-cm^3 soil aliquant per microplot were washed free of soil, fixed in formal-acetic-alcohol (6.1.20) for 48 h, and stained in cold acid fuchsin-lactophenol (17). Forty hours later, roots were destained in lactophenol, mounted between two microscope slides and observed under a compound microscope (100 ×). The number of nematodes inside the roots was determined. Tobacco roots collected at 60, 90, and 104 d after inoculation were processed as in 1978. Average soil temperatures and precipitation were determined as in 1978.

RESULTS

In the 1978 test, foliar symptoms of *M. javanica,* but not *M. incognita,* damage were evident 5 wk after transplanting into the medium (16 nematodes/100 cm^3 of soil) and high (64 nematodes/100 cm^3 of soil) initial inoculum density soils. Stunting was obvious on all plants and the lower leaves displayed necrosis along their margins (rimfiring). Before the first harvest, rimfiring symptoms were evident on several plants inoculated with the low P_i (four nematodes/100 cm^3 of soil) of *M. javanica.* By 124 d after transplanting into infested soil, two plants from the medium P_i of *M. javanica* and ten plants from the high P_i had died. Roots of those plants were heavily galled and deteriorated. Of the plants inoculated with *M. incognita,* only those inoculated with the high P_i showed widespread symptoms (stunting and rimfiring) of nematode damage. Occasionally stunted plants were found at the low and medium P_i of *M. incognita.* Similar results were obtained in 1979, except disease symptoms were more pronounced.

In both years, *M. javanica* significantly reduced tobacco leaf yield at each of the three inoculum levels as compared to the control or tobacco plants inoculated with the same levels of *M. incognita* (Table 1). Linear regression analysis of P_i and tobacco yield for the 1978 season showed a 13, 25, and 58% yield suppression at the low, medium, and high levels of *M. javanica,* respectively (Figure 1). At the low P_i of *M. incognita,* yield of tobacco plants was 3% greater than the control plants. At the medium and high P_i of *M. incognita,* suppression of tobacco leaf yield was 4 and 35%, respectively. In 1979, regression analyses of P_i and tobacco yield showed a 31, 39, and 70% yield suppression at the low,

Table 1. Effects of *Meloidogyne incognita* and *M. javanica* on tobacco 'McNair 944' leaf yield at three initial inoculum levels (P_i).

	P_i (nematodes/100 cm^3 soil)	Yield* (g/microplot) 1978	1979
Control	0	380 a†	355 a
M. incognita	4	392 a	340 a
M. javanica	4	272 b	273 b
M. incognita	16	270 b	288 b
M. javanica	16	217 c	180 c
M. incognita	64	292 b	250 b
M. javanica	64	174 c	112 c

*Average dry weight of leaves from two plants/microplot per treatment.

†Grouped means followed by a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

medium, and high P_i of *M. javanica,* respectively. At the low, medium, and high P_i of *M. incognita,* suppression of tobacco leaf yields were 9, 13, and 34%, respectively.

Reproduction of nematodes varied in the 1978 and 1979 tests, but similar trends were observed (Table 2). In 1978, the numbers of *M. incognita* eggs and larvae extracted from the medium and high P_i soils after 124 d were twice those of *M. javanica.* No differences in reproduction of the two species occurred at the low P_i or 60 d after inoculation at any inoculum level. In 1979, more larvae of *M. javanica* than of *M. incognita* were extracted from the soil at the low, medium, and high P_i 30 d after inoculation. No differences in reproduction of the two species occurred at the medium and high P_i 60 d after inoculation or at the low and medium P_i 104 d after inoculation. Reproduction of the two species did not differ 90 d after inoculation at any inoculum level. Numbers of *M. javanica* larvae were four times those of *M. incognita* 60 d after inoculation at the low P_i. At the end of the season, numbers of *M. incognita* larvae were three times those of *M. javanica* at the high P_i.

Coefficients of correlation between (i) root-gall index and P_i, (ii) yield and root-gall index, and (iii) yield and P_i were determined for plants infected with *M. incognita* and *M. javanica* in 1978 and 1979

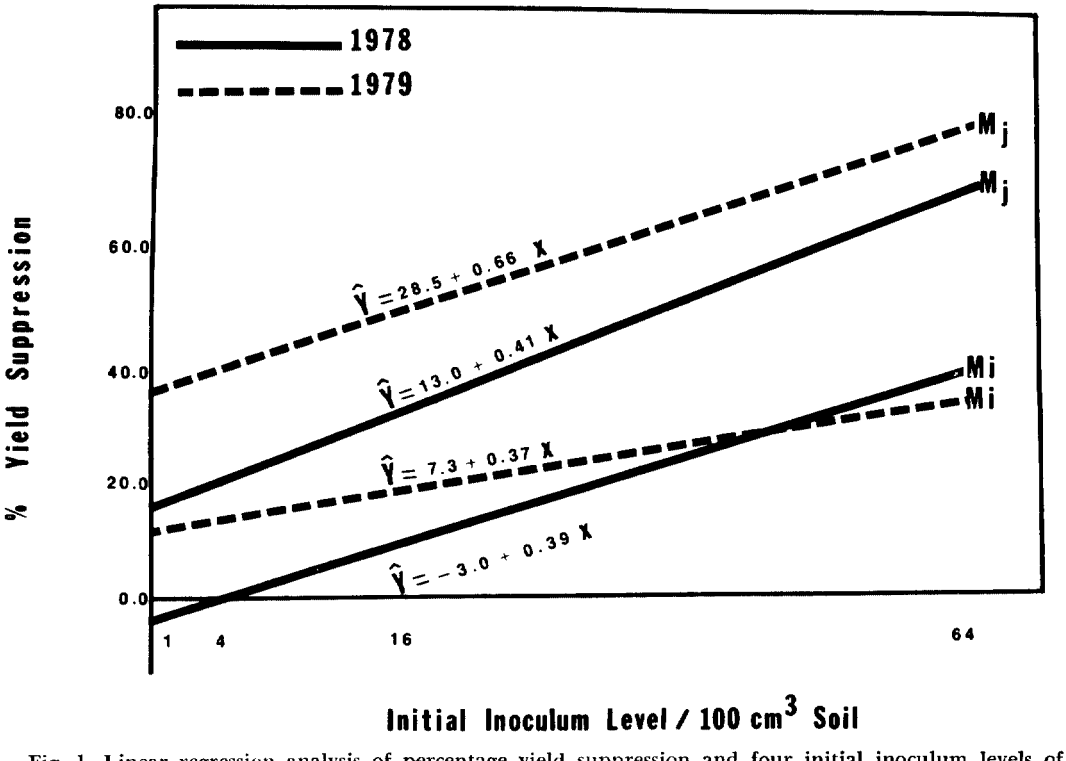

Fig. 1. Linear regression analysis of percentage yield suppression and four initial inoculum levels of *Meloidogyne incognita* (M.i.) and *M. javanica* (M.j.) on tobacco 'McNair 944.'

Table 2. Reproduction of *Meloidogyne incognita* and *M. javanica* on tobacco 'McNair 944' at three initial inoculum levels (P_i).

	P_i (nematodes/100 cm³ soil)	Nematodes/100 cm³ soil*					
		1978†		1979‡			
		60 d	124 d	30 d	60 d	90 d	104 d
Control	0	84 b§	98 c	0 c	0 c	0 c	0 c
M. incognita	4	106 a	1299 a	30 b	164 b	1210 a	1847 a
M. javanica	4	118 a	923 a	103 a	609 a	1535 a	1552 a
M. incognita	16	128 a	1874 a	69 b	413 a	1530 a	1670 a
M. javanica	16	370 a	903 b	226 a	323 a	1127 a	1464 a
M. incognita	64	250 a	3237 a	198 a	587 a	826 a	1284 a
M. javanica	64	350 a	1475 b	460 a	799 a	515 a	476 b

*Include nematodes present in root and soil in 100 cm³ of soil.
†Average of five soil cores/microplot per treatment.
‡Average of four soil cores/microplot per treatment.
§Grouped means and control followed by a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

(Table 3). Except for *M. javanica* in 1979, the correlations between root-gall index and the P_i were low and not significant. Similarly, yield and root-gall index did not appear to be correlated, except galling caused by *M. javanica* in 1979. Yield and P_i were significantly, and negatively, correlated for both nematode species in both sampling years.

In 1978, the average soil temperatures during April, May, June, and July were 26 C, 29 C, 34 C, and 34 C, respectively. The average precipitation for those same months was 0.22, 0.32, 0.21, and 0.84 cm, respectively. In 1979, the average soil temperatures during April, May, June, and July were 27, 30, 32, and 33 C, respectively. The average precipitation during those months was 0.41, 0.30, 0.16, and 0.76 cm, respectively.

DISCUSSION

Microplot test results provided useful information on the effects of initial inoculum densities of *M. incognita* and *M. javanica* on tobacco yield in Florida. Linear regression equations most closely fit data on P_i and percentage yield suppression with *M. incognita,* although quadratic equations provide better data for *M. javanica.* This is thought to be a result from the greater and early effects of *M. javanica* on tobacco growth and later effects of *M. incognita* which allowed some plant compensation for nematode damage. For comparative purposes, linear equations were used and initial injury levels were arbitrarily set at the nematode P_i which caused 7% plant yield suppression. When using these criteria,

Table 3. Correlation coefficients of three variables with *Meloidogyne incognita* and *M. javanica* during 1978 and 1979 field tests on tobacco 'McNair 944.'

Variables	*M. incognita*		*M. javanica*	
	1978	1979	1978	1979
Root-gall index vs P_i*	0.19	0.28	0.37	0.71**†
Yield vs root-gall index	−0.12	−0.30	−0.26	−0.74**
Yield vs P_i	−0.77**	−0.73**	−0.68**	−0.77**

*P_i = initial inoculum level.
†Column means followed by ** are significant at 1% level, according to the test of significance from Fisher and Yates.

initial injury levels were calculated to occur with fewer than one egg or second-stage *M. javanica* larva per 100 cm^3 of soil for both test years; eight eggs or second-stage *M. incognita* larvae were required in 1978, but only one in 1979. These low P_i's reflect the highly aggressive nature of both nematode species on flue-cured tobacco as compared to other crop plants (3,5). These data do not include effects on tobacco leaf quality which would probably further lower all injury levels.

Because of the low number of nematodes necessary to cause tobacco damage, problems arise in correctly determining populations in the field. It is suspected that spring populations of these nematodes are at or below limits that can be detected with currently available sampling and extraction procedures. Thus, fall sampling would be more useful than spring nematode counts (23). In addition, injury levels differed considerably between the 1978 and 1979 growing seasons, further indicating the need to assign ranges in initial injury level determination. It is thought that in these tests plant response to environmental variables were responsible for most of the yield variation between years. For example, early season soil temperatures and precipitation were higher in 1979 than in 1978.

Populations of both nematodes increased rapidly over initial inoculum levels. Early in the two tests (30–60 d after inoculation), however, numbers of *M. javanica* were usually greater than those of *M. incognita*. As the season progressed, numbers of *M. javanica* either declined or did not increase in proportion to those of *M. incognita*. As evidenced from comparative yield data and foliar symptoms, greater invasion and reproduction of *M. javanica* early in the season apparently did not allow the tobacco root systems to develop. Brodie and Dukes (8) similarly found that *M. javanica* caused the most damage early in the tobacco growing season. Conversely, invasion and reproduction of *M. incognita* occurred at a lower rate early in the season and allowed the plants to develop more normally. This situation is analogous to that of Seinhorst's hypothesis (22) in which he states that the amount of damage caused by a nematode is related to the preplant nematode density and the amount of exposed tissue not attacked. Differences in reproduction of these two nematodes have been found in growth chamber experiments, but reasons for these differences are unclear (1). Preliminary data, however, indicate that *M. javanica* larvae locate and more readily invade tobacco roots than do *M. incognita* at temperatures approximating those in the first 2 months of the growing season.

Microplots combine the advantages of ease of handling, close approximation to field conditions, the possibility of incorporating several nematode densities, and the establishment of fairly uniform densities within a treatment (9,14,15). It is a useful method for assessing injury levels of a crop exposed to several preplant densities of plant-parasitic nematodes. Microplot data, however, are only an approximation of minimal damage densities (5). Homogeneity in nematode populations does not occur in the field, and calculated damaging densities under field conditions can only be approximated. These data, however, suggest growers should be aware that the presence of even a very low P_i of *M. javanica* in a tobacco field will cause reduction in yield. Since no resistant cultivars are available, other control measures must be optimally integrated to prevent *M. javanica*-induced losses in Florida tobacco fields.

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