Comparative Studies on Root Invasion, Root Galling, and Fecundity of Three *Meloidogyne* spp. on a Susceptible Tobacco Cultivar[1]

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Abstract: Root invasion, root galling, and fecundity of *Meloidogyne javanica, M. arenaria,* and *M. incognita* on tobacco was compared in greenhouse and controlled environment experiments. Significantly more *M. javanica* than *M. arenaria* or *M. incognita* larvae were found in tobacco roots at 2, 4, and 6 d after inoculation. Eight days after inoculation there were significantly more *M. arenaria* and *M. javanica* than *M. incognita* larvae. Ten days after inoculation no significant differences were found among the three *Meloidogyne* species inside the roots. Galls induced by a single larva or several larvae of *M. javanica* were significantly larger than galls induced by *M. incognita; M. arenaria* galls were intermediate in size. Only slight differences in numbers of egg masses or numbers of eggs produced by the three *Meloidogyne* species were observed up to 35 d after inoculation. *Key words: Meloidogyne arenaria, M. incognita, M. javanica,* root invasion, fecundity, gall size, tobacco.

Extensive studies have been conducted on the pathogenicity and population development of plant-parasitic nematodes on tobacco (3,6,10). The degree of host damage and nematode reproduction has been related to factors such as initial population density (8), the nematode species (4), plant cultivar (3), and environmental conditions (10). Studies comparing the effects of two or more nematode species of the same genus on tobacco have received less attention. Southards (15) found that *Pratylenchus scribneri* and *P. zeae* did not reproduce well on tobacco, but *P. brachyurus* reproduced mod-

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erately well. Barker (2) observed differences in pathogenicity of various *Meloidogyne* species on tobacco. His results showed *M. javanica* was the most damaging, followed in order by *M. arenaria, M. incognita,* and *M. hapla.* Similar results were obtained by Arens (1) when working with *M. javanica* and *M. incognita.* She suggested the greater reproduction of *M. javanica* early in the season may have contributed to a greater yield reduction. Several factors may be postulated for these differences in nematode reproduction and damage to tobacco. These include (i) greater ability of one species to locate and invade host roots, (ii) differences in infection sites, (iii) greater inherent reproductive capacities, (iv) differences in root galling on the host plant, and (v) more favorable environmental conditions. The present studies were designed to investigate three of these factors: root invasion, fecundity, and gall size on tobacco roots inoculated with *M. javanica, M. incognita,* and *M. arenaria.*

MATERIALS AND METHODS

Greenhouse experiment: Fifty-day-old tobacco (*Nicotiana tabacum* L.) cv. McNair 944 seedlings were transplanted into 26-cm-d pots containing 5,200 cm^3 of methyl bromide fumigated (988.8 kg/ha) Lakeland fine sand soil (93.1% sand, 3.9% silt, and 3.0% clay). One week after transplanting, three groups of 15 seedlings each were inoculated with 4, 16, and 64 *M. arenaria, M. incognita,* or *M. javanica* nematode eggs and/or second-stage larvae (L_2) per 100 cm^3 of soil. Noninoculated plants served as controls. Eggs and L_2 were extracted from tomato (*Lycopersicon esculentum* Mill.) cv. Rutgers using 1% sodium hypochlorite (9). A 5-ml syringe was used to place the inoculum into three holes, each 1 cm from the base of the plant and 9 cm deep in the soil. The pots were placed on a greenhouse bench in a completely randomized design. Tobacco plants were fertilized weekly with a 0.5% solution of Peters Special[R] (20-20-20) fertilizer (Robert B. Peters Co. Inc., Allentown, Pennsylvania) and watered as needed. Soil temperature during the experiment averaged 33 C.

Twenty, forty, and sixty days after inoculation, the roots from five pots per treatment were collected, washed free of soil, and fixed in formal-acetic alcohol (6:1:20) for 48 h. The roots were stained in cold acid fuchsin-lactophenol (11). A caliper vernier was used to measure the length and width of 50 galls from each root system. Individual galls were dissected under a stereoscopic microscope at 80 × to determine the number of L_2 inside the galls. Also, the number of nematodes inside 0.5 g of each root system was determined.

Controlled environment experiment: Fifty-day-old tobacco seedlings were transplanted into 5-cm-d pots containing 200 cm^3 of soil. The pots were placed in a controlled environment chamber programmed for a 12 h photoperiod (5,000 foot candles). The average soil temperature was 26 C and intended to simulate early season (April, May) field conditions at a soil depth of 10 cm. The statistical design and fertilization rates were as described above. One week after transplanting, two experiments were initiated, each using seedlings randomly allocated into three groups of 50 each. Inoculation procedures were as described above, except the inoculum was placed into two holes, each 1 cm from the plant and 2.5 cm deep.

Tobacco roots inoculated with 250 freshly hatched L_2 of *M. javanica, M. arenaria,* and *M. incognita* were randomly harvested 2, 4, 6, 8, and 10 d after inoculation. Root systems of 10 plants per nematode species were collected at each date and fixed and stained as above. The stained roots were mounted between two microscope slides, and the number of nematodes inside determined under 100 × magnification.

Plants inoculated with 600 L_2 of the three *Meloidogyne* species were carefully removed from pots 48 h after inoculation, washed free of soil, and transplanted into 13-cm-d pots containing 500 cm^3 of soil. Roots from 10 tobacco plants per species were collected separately and weighed 20, 25, 30, and 35 d after inoculation. Fifty randomly selected egg masses per treatment were individually removed from the roots and placed in 0.5% sodium hypochlorite for 2 min to free the eggs from the gelatinous matrix. The number of eggs in each egg mass was counted under a stereoscopic microscope at 80 ×. Nematodes in the root

systems and size of root galls were determined as above. The experiment was repeated for gall size measurements, but fecundity data were not taken.

RESULTS

Galls induced by *M. javanica* and *M. arenaria* from the greenhouse experiment were larger than those of *M. incognita* at the three sampling dates (Table 1). *M. arenaria* and *M. javanica* galls did not differ in size 20 and 60 d after inoculation, but at 40 d galls produced by *M. javanica* were larger than those of *M. arenaria*. The total number of *M. javanica* nematodes inside the roots was greater than those of *M. arenaria* or *M. incognita* at all harvest dates. Also, the number of *M. arenaria* inside the roots was always greater than that of *M. incognita*. The total number of *M. javanica* L_2 dissected from 50 galls was greater than the number dissected from *M. arenaria* or *M. incognita* from roots at all harvest dates. The number of *M. arenaria* L_2 was greater than that of *M. incognita* after 20 d but not different at 40 or 60 d after inoculation.

The three *Meloidogyne* species differed in rate of root invasion. Significantly more *M. javanica* than *M. arenaria* or *M. incognita* were found inside the roots at 2, 4, or 6 d after inoculation (Table 2). The number of *M. arenaria* L_2 was greater than that of *M. incognita* at 4 and 8 d, but not at 2 and 6 d after inoculation. After 10 d, nematode numbers inside the roots were similar among the three *Meloidogyne* species.

Enlarged *M. javanica* L_2 were first observed inside the tobacco roots 6 d after inoculation. After 8 and 10 d, a higher percentage of swollen *M. javanica* L_2 than *M. incognita* and *M. arenaria* was found (Table 3). Swollen *M. arenaria* L_2 and *M. incognita* were first observed 8 d after inoculation with little increase between 8 and 10 d after inoculation.

Gall sizes induced by single females differed among the three *Meloidogyne* species.

Table 1. Average gall size, number of nematodes per root system, and number of second-stage larvae (L_2) dissected from inside 50 galls of tobacco inoculated with three *Meloidogyne* spp.

	Days after inoculation*		
	20	40	60
	Gall size (mm²)†		
M. javanica	4.71 a‡	32.73 a	19.19 a
M. arenaria	3.90 a	22.45 b	17.46 a
M. incognita	0.92 b	14.17 c	11.66 b
	No. nematodes in 0.5 g of root		
M. javanica	40 a	274 a	133 a
M. arenaria	21 b	130 b	72 b
M. incognita	4 c	40 c	35 c
	No. L_2/50 galls		
M. javanica	10 a	69 a	23 a
M. arenaria	5 b	33 b	13 b
M. incognita	1 c	10 b	6 b

*Inoculum density is a combination of 4, 16, and 64 nematode eggs and/or L_2/100 cm³ of soil.
†Gall size determined by length × width.
‡Means followed by a common letter are not significantly different at the 5% level according to Duncan's multiple-range test.

Table 2. Number of nematodes found inside the roots of tobacco plants at five intervals after inoculation with three *Meloidogyne* spp.

Days after inoculation	No. of nematodes inside of roots*		
	M. javanica	*M. arenaria*	*M. incognita*
2	93 a†	5 b	3 b
4	128 a	27 b	8 c
6	124 a	41 b	46 b
8	113 a	108 a	63 b
10	108 a	95 a	93 a

*Averages of 10 root systems/treatment.
†Means across columns followed by a common letter are not significantly different at the 5% level according to Duncan's multiple-range test.

Table 3. Percentage of swollen second-stage larvae (L_2) inside the roots of tobacco plants after inoculation with three *Meloidogyne* spp.

	Days after inoculation	% swollen L_2/root system*
M. javanica	8	5 a†
M. arenaria	8	2 b
M. incognita	8	2 b
M. javanica	10	11 a
M. arenaria	10	2 b
M. incognita	10	3 b

*Averages of 10 root systems/treatment.
†Means followed by a common letter are not significantly different at the 5% level according to Duncan's multiple-range test.

Galls induced by single *M. javanica* were larger than those formed by *M. incognita* at all observation dates (Table 4). Variations in statistically significant gall sizes were found among the three nematodes although largest galls were found with *M. javanica,* followed by *M. arenaria* and then *M. incognita.*

The three *Meloidogyne* species differed in fecundity. *M. arenaria* or *M. javanica* produced more eggs than *M. incognita* 20 and 25 d after inoculation, respectively (Table 5). The fecundity of the three *Meloidogyne* species was similar 30 d after inoculation. After 35 d, *M. javanica* and *M. incognita* produced more eggs than *M. arenaria.*

Differences in the percentage of females with egg masses were only found 20 and 35 d after inoculation (Table 5). At 20 d, the percentage of *M. javanica* with egg masses was greater than that of *M. incognita.* After 35 d, more egg masses of *M. javanica* and *M. arenaria* were found than of *M. incognita.*

Table 5. A comparison on number of eggs and percentage of egg masses produced by females of three *Meloidogyne* spp.

	Days after inoculation	No. of eggs* per female	% females† with egg masses
M. javanica	20	86 ab‡	73 a
M. arenaria	20	92 a	67 ab
M. incognita	20	70 b	57 b
M. javanica	25	270 a	90 a
M. arenaria	25	259 ab	95 a
M. incognita	25	226 b	91 a
M. javanica	30	195 a	98 a
M. arenaria	30	172 a	100 a
M. incognita	30	265 a	94 a
M. javanica	35	265 a	92 a
M. arenaria	35	208 b	80 a
M. incognita	35	274 a	42 b

*Averages of 50 egg masses/treatment.

†Percentage of females with egg masses based on the average number of females over females with egg masses in five tobacco plant root systems/treatment.

‡Means followed by a common letter are not significantly different at the 5% level according to Duncan's multiple-range test.

DISCUSSION

Differences in aggressiveness and population development of the three *Meloidogyne* species on tobacco may be primarily due to differences in the rate of root invasion and the size of galls induced by these nematodes. These experiments indicated *M. javanica* invaded tobacco roots in greater numbers and produced larger galls than did *M. incognita; M. arenaria* was generally between the two extremes. Field microplot data of Barker (2,3) and Arens (1) compare favorably with these results.

Table 4. Average size of 50 single female galls (mm^2)* produced by three *Meloidogyne* spp. on tobacco.

	Days after inoculation†			
	20	25	30	35
M. javanica	1.08 a‡	1.01 a	1.24 a	1.37 a
M. arenaria	0.92 b	0.91 ab	0.84 b	0.87 b
M. incognita	0.83 b	0.86 b	0.67 c	0.72 c

*Gall size determined by length × width.

†Inoculum density of 300 second-stage larvae/100 cm^3 of soil.

‡Means followed by a common letter are not significantly different at 5% level according to Duncan's multiple-range test.

In the greenhouse experiment, data indicated gall size was correlated with numbers of nematodes inside the tobacco roots. These data suggest that gall size primarily depends upon the numbers of nematodes present. Similar results were attained by McClure and Viglierchio (12) who found that a fourfold increase in the inoculum level of *M. incognita* produced a ninefold increase in gall size. Nevertheless, in cases where galls were induced by a single nematode (controlled environment experiments) the same pattern of gall size was attained: *M. javanica* > *M. arenaria* > *M. incognita.* These gall size differences indicated *M. javanica* may affect the physiology of the tobacco plant to a greater extent than do these other two species. A larger gall could increase the amount of phloem and xylem disruptions and also increase the area in which secondary microorganisms could invade (7,13). Subsequently, damage from this nematode would be expected to be greater.

As in the case of gall size, root invasion of *M. javanica* was found to be greater than

that of the other two species. A number of factors are postulated for differences in larval invasion among root-knot nematode species. Among these factors, infection site, biological differences in species vigor, temperature effects, and host preferences appear to be the most important (7,16). Sayre (14) suggested that availability for root sites of *M. incognita* infection is limited by the increase in inoculum level. Vrain et al. (18) noted that the rate of invasion and reproduction of *M. hapla* and *M. incognita* in clover roots was not only a function of temperature, but it was influenced also by the condition of the host plants. In the present investigation, low inoculum levels were maintained but the three *Meloidogyne* differed in their ability to invade tobacco roots. The greater ability of *M. javanica,* as compared to *M. arenaria* or *M. incognita,* to locate and invade tobacco roots may be a significant factor in its more aggressive nature on tobacco. In the field, larvae of *M. javanica* may invade the root system and produce more galls at a faster rate than the other two species. This could have the effect of increasing the total early season reproduction and damage caused by *M. javanica* as compared to *M. arenaria* or *M. incognita.*

Temperature requirements for invasion and reproduction differs significantly among root-knot nematode species, and their temperature optima varies for different host-parasite combinations (5,17). All three *Meloidogyne* species are known to occur in Florida tobacco fields. The temperature under which the present experiments were conducted simulated field conditions, but they revealed little differences in fecundity of the three *Meloidogyne* species. Our reproductive data represent only one test, however, and fecundity cannot be ruled out as being partially responsible for differences in aggressiveness and populations of *M. javanica, M. arenaria,* and *M. incognita.* These data, however, indicated that fecundity of these nematodes may not be a major factor in the plant response, at least in the first 60 d of the tobacco growing season. Differences in aggressiveness shown by the three *Meloidogyne* species appeared to be related primarily to their ability to invade and induce galls.

LITERATURE CITED

1. Arens, M. L. 1979. Reasons for differential aggressiveness of three Meloidogyne spp. Goeldi, 1887 on tobacco (Nicotiana tabacum L.), and injury levels of two of these species. M. S. Thesis, University of Florida, Gainesville.

2. Barker, K. R. 1977. Yield losses of tobacco caused by four species of Meloidogyne. J. Nematol. 9:263 (Abstr.).

3. Barker, K. R. 1978. Relative sensitivity of flue-cured tobacco cultivars to four species of Meloidogyne. J. Nematol. 10:281-282 (Abstr.).

4. Barker, K. R., and T. H. A. Olthof. 1976. Relationships between nematode population densities and crop responses. Ann. Rev. Phytopathol. 14:327-353.

5. Bird, A. F., and H. R. Wallace. 1965. The influence of temperature on Meloidogyne hapla and M. javanica. Nematologica 11:581-589.

6. Brodie, B. B., and P. D. Dukes. 1972. The relationship between tobacco yield and time of infection with Meloidogyne javanica. J. Nematol. 4: 80-83.

7. Endo, B. Y. 1975. Pathogenesis of nematode-infected plants. Ann. Rev. Phytopathol. 13:213-237.

8. Ferris, H. 1974. Correlation of tobacco yield, value, and root-knot index with early-to-midseason and postharvest Meloidogyne population densities. J. Nematol. 6:75-81.

9. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of Meloidogyne spp. including a new technique. Plant Dis. Reptr. 57:1025-1028.

10. Lucas, G. B. 1975. Diseases of tobacco. Biological Consulting Associates, Raleigh, North Carolina.

11. McBeth, C. W., A. L. Taylor, and A. L. Smith. 1941. Note on staining nematodes in root tissues. Proc. Helminthol. Soc. Wash. 8:26.

12. McClure, M. A., and D. R. Viglierchio. 1966. Penetration of Meloidogyne incognita in relation to growth and nutrition of sterile, excised cucumber roots. Nematologica 12:237-247.

13. Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Meded. Landhouwhogesch. Wageningen, 66-4.

14. Sayre, R. M. 1958. Plant-tissue culture as a tool in the study of the physiology of the root-knot nematode, Meloidogyne incognita Chitwood, Ph.D. Thesis, University of Nebraska, Lincoln.

15. Southards, C. J. 1966. Host-parasite relations of the lesion nematodes, P. brachyurus, P. zeae, and P. scribneri, and flue-cured tobacco. Plant Dis. Reptr. 26:4164-4165.

16. Thomason, I. J. 1962. Reaction of cereals and sudan grass to Meloidogyne spp. and the relation of soil temperature to Meloidogyne javanica population. Phytopathology 52:787-791.

17. Thomason, I. J., and B. Lear. 1961. Rate of reproduction of Meloidogyne spp. as influenced by soil temperature. Phytopathology 51:520-524.

18. Vrain, T. C., K. R. Barker, and G. I. Holtzman. 1978. Influence of low temperature on rate of development of Meloidogyne incognita and M. hapla larvae. J. Nematol. 10:166-171.