Meloidogyne graminis and Meloidogyne spp. on Zoysia; Infection, Reproduction, Disease Development, and Control

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ABSTRACT

Root growth of three cultivars of zoysia and bermudagrass inoculated with *Meloidogyne graminis* in the greenhouse was adversely affected, but 4 and 7 mo after inoculation the fresh root and top wt of inoculated plants did not differ significantly from uninoculated plants. *M. graminis* larvae penetrated and infected *Zoysia japonica,* 'Meyer' and 'Emerald' zoysia, and 'Sunturf' bermudagrass. Optimum temp for penetration and development of *M. graminis* on Meyer zoysia was 28 C. Larvae of *M. incognita* and *M. hapla* penetrated and infected the three cultivars of zoysia. Histopathology of infection by *M. incognita* and *M. hapla,* including giant cell formation and other host tissue reactions, was similar. Hypertrophy of cells appeared to be the primary means means of tissue expansion surrounding *M. graminis* females, whereas hyperplasia was much more evident around feeding sites of *M. incognita* and *M. hapla* females. Hot water treatment of bare-rooted zoysia plants infected with *M. graminis* for 10 min at 50 C eradicated the nematode.

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Meloidogyne graminis is a nematode parasite of turf in the southern part of the U.S. Damage from the nematode is evidenced by circular areas of dead or dying grass, and plants at the margins of affected areas may show various stages of chlorosis. *M. graminis* was originally found by Sledge (15) in 1959 on St. Augustinegrass in Florida, and Van Weerdt et al. (17) subsequently reported the occurrence of the nematode. The nematode has been found occurring in the field on St. Augustine, bermuda and zoysia grasses, and other grasses have been reported as hosts as a result of inoculation tests (1, 4, 5, 10, 13, 15, 16, 18). *M. graminis* was first found in Arkansas on zoysia in 1967 (R. D. Riggs, unpublished) and has increased in prevalence within the state.

Infection of St. Augustine (11, 12) and bermuda (6) grasses by *M. graminis* has been thoroughly investigated, and some observations have been made on infection of zoysia (4) by this nematode. This study was made to observe the developmental cycle of *M. graminis* on different cultivars of zoysia under controlled conditions, to compare its developmental cycle and infection processes with two other *Meloidogyne* species, to investigate root injury, and to determine feasibility of hot

water treatment of roots to control the nematode on grass for nursery plantings.

MATERIALS AND METHODS.—*Meloidogyne graminis* (Sledge & Golden) Whitehead was maintained in the greenhouse on Meyer zoysia. *M. incognita* (Kofoid & White) Chitwood and *M. hapla* Chitwood were maintained on Rutgers tomato. Inoculum consisted either of counted larvae hatched from egg masses in watch glasses, or egg masses from source plants when exact inoculum levels were not necessary. The larvae or egg masses were added in 1-2 ml of water to the surface sand around potted plants. Pots then were watered to moisten the sand and to wash-in the nematodes.

The zoysia grasses used in the tests were *Zoysia japonica* Steud., *Z. japonica* 'Meyer', and *Z. japonica* × *Z. tenuifolia* Willd. ex Trin. 'Emerald.' Bermudagrass, *Cynodon magennisii* Hurcombe 'Sunturf' was used in one test for comparison purposes. Plants of the grasses were obtained from excised tips of rapidly elongating stolons which were rooted in sand after stolons were determined to be free from nematodes. The grasses were grown in the greenhouse in sterilized sand in clay pots or plastic cups and were watered bi-weekly with a balanced fertilizer solution.

In some experiments, nematodes were stained in situ with bromphenol blue (8). Nematodes were collected by standard methods.

Pathogenicity tests.—The effects of three population levels of *M. graminis* on the growth of *Z. japonica,* Meyer zoysia, and Emerald zoysia and the intermediate level on Sunturf bermudagrass was determined. Plants of each zoysia cultivar growing in 8.5-cm diam clay pots without bottom holes were inoculated with 0, 100, 200, and 400 larvae per pot; bermudagrass was inoculated with 0 and 200 larvae per pot. There were ten pots per treatment. Fresh wts of roots and tops were recorded 4 and 7 mo after inoculation, and the results subjected to analysis of variance. Nematode-infected and noninfected roots were also compared visually for macroscopic differences.

Interaction of temperature and time on M. graminis penetration and development.—Equal numbers of small Meyer zoysia plants in plastic cups were placed in growth chambers at 16, 22, 28, and 34 C and, after 2 days 200 *M. graminis* larvae were added per cup. Roots were stained with bromphenol blue after 12, 24, 36, 48, and 60 h, and after 5, 7, 14, and 36 days when observations were made for penetration and development of the nematodes.

Susceptibility of zoysia cultivars to three Meloidogyne spp.—Eight pots each of *Z. japonica,* Meyer zoysia, and Emerald zoysia were infested with 200 ± 10 larvae of *M. graminis, M. incognita,* or *M. hapla*. Roots of two plants from each treatment were stained with bromphenol blue 7, 14, 33, and 38 days after inoculation and observed for the number of nematodes that had parasitized each root, the stage(s) of development, and effects of the nematodes on the root.

Histopathology.—Development of *M. graminis, M. incognita,* and *M. hapla* and their cellular pathology was compared on Meyer zoysia. Three groups of 20 plants were inoculated with 250 larvae of each nematode species. Two plants from each group were examined 1, 2, 7, 14, 20, 24, 28, 32, and 36 days after the nematodes were added to the cups. The 1-, 2-, and 7-day samples were grown in 10-ml plastic cups, and the others were grown in 100-ml plastic cups. Roots were stained with bromphenol blue, examined, and selected roots containing nematodes were dehydrated by the tertiary butyl alcohol-paraffin oil method and embedded in paraffin. Longitudinal and transverse sections were cut at a thickness of 8-10 μm, stained with safranin and fast green, and examined by light microscopy.

Hot water treatment of nematode-infected roots.—Sprigs of Meyer zoysia heavily infected with *M. graminis* were washed free of sand and submerged in water baths at 40, 42, 44, 46, 48, and 50 C for 10 min. Five springs were treated at each temp, submerged in cool tap water for 15 min, then potted in sterilized sand. After 1 wk a healthy spring with nematode-free roots was transplanted to each pot as an indicator plant to help determine whether viable nematodes were present. Five weeks later, the treated and indicator plants were observed and their roots stained. Sand and wash water were collected from each plant and checked for nematodes. The test was repeated using one-degree temp intervals from 45-55 C for 10 min. Eight sprigs were treated at each temp between 48 and 52 C, and five sprigs were used for the other temp. Observations and determinations of the presence of nematodes were made after 2 mo.

RESULTS.—*Pathogenicity tests.*—Lateral roots of zoysia and bermudagrass plants infected with *M. graminis* were discolored and sometimes necrotic, and growth was reduced. Growth of primary roots was also inhibited. Leaves of plants parasitized by *M. graminis* became yellowed about 1 wk after inoculation, but most regained their green color after 6-8 wk. Four and 7 mo after inoculation there were no significant differences between the fresh root and top wts of control and inoculated zoysia and bermudagrass plants.

Interaction of temperature and time on M. graminis penetration and development.—*M. graminis* larvae penetrated and developed in roots of all Meyer zoysia plants grown at 16, 22, 28, and 34 C. Partial entry of larvae was observed 24 h after inoculation at 34 C, and after 36 h, larvae were well embedded in the roots. At 28 C, total penetration of larvae was not observed until 48 h after inoculation. Penetration had occurred in some plants grown at 16 and 22 C at 48 and 60 h after inoculation, and all plants contained *M. graminis* larvae 5 days after inoculation.

Optimum temp for *M. graminis* development was 28 C. Seven days after inoculation nematodes on plants at 28 and 34 appeared to be at a similar stage of development, while those at 22 C were established, but less well-developed. Fourteen days after inoculation enlarging females were most developed on plants at 28 C and least developed at 34 C. After 36 days, the greatest number of fully developed egg masses were found on plants grown at 28 C. A larger number of fully developed egg masses were observed at 22 than at 34 C.

Susceptibility of zoysia to three Meloidogyne spp.—*M. graminis, M. incognita* and *M. hapla* penetrated and developed in roots of *Z. japonica,* Meyer and Emerald zoysias (Table 1). *M. graminis* penetrated more successfully than *M. incognita,* and penetration and development of *M. hapla* was erratic. At greenhouse temp of approximately 30 C, egg masses of *M. graminis* and *M. incognita* were observed 33 days after inoculation, and

egg masses of *M. hapla* were found 38 days after inoculation. *M. incognita* and *M. hapla* females produced galls and were frequently found on lateral roots. Only an occasional, slight root swelling was observed at the infection site of *M. graminis* females, mostly on primary roots.

Histopathology.—*M. graminis* larvae penetrated Meyer zoysia roots within 48 h after inoculation at greenhouse temp of approximately 30 C. The greatest number of larvae penetrated just above the root cap in the meristemic region of the root tip. Larvae migrated inter- and intracellularly through the cortex, assuming a final position parallel with and near the central cylinder (Fig. 1-A). Larvae were observed in groups in the cortex, and it appeared they followed the same path of penetration. The nematodes were always found with the anterior end oriented toward the root tip. After the larvae became sedentary, they fed on cells in the outer periphery of the central cylinder with only the esophageal region extended into the stele.

Seven days after inoculation larvae were cigar-shaped, ca. 450 μm in length and 45 μm in diam. At 14 days they were 85 μm in diam (Fig. 1-B). Twenty days after inoculation, females were 450-500 μm long and 250 μm in diam, and after 24 days had reached their maximum growth of ca. 750 × 375 μm. Egg masses were present after 24 days, and coiled larvae were seen in eggs 32 days after inoculation. Thirty-six days after inoculation, second-stage larvae were observed in the cortex, indicating that eggs had hatched and that larvae had penetrated the roots.

The esophageal region of the mature female extended into the central cylinder of the root, with the body embedded in the cortex outside the endodermis. Egg masses extended 300-500 μm behind the female, and most of the masses protruded to the outside of the root. Egg masses contained an estimated 150-350 eggs.

There was definite evidence of host reaction to the presence of *M. graminis* larvae in roots fixed 48 h after inoculation. Cells immediately surrounding the anterior of the larvae were hypertrophied, and had dense cytoplasm which contained abnormally large nuclei and nucleoli. The cell enlargement was the initial reaction in the formation of giant cells.

Giant cells were formed in the procambium around the anterior of nematodes, always in the central cylinder (Fig. 1-C). The protoplasm of giant cells examined 24 days after inoculation appeared to be homogenous (Fig. 1-D). Some cells extended as much as 500 μm in either direction along the root axis from the tip of the nematode. Each *M. graminis* female was surrounded by 4-7 multinucleate giant cells, and when two females fed in the same general area, 8-10 giant cells were present. Each giant cell contained 15-25 large nuclei ca. 13-15 μm in diam, with large nucleoli averaging 5.2 μm in diam. The giant cells were surrounded by an increased number of tracheids, and the secondary thickenings of the pericycle and endodermis were reduced in areas adjacent to infection sites. Hypertrophy and hyperplasia of the pericycle and endodermis were observed in some roots. In small roots, or in areas of multiple infection, the vascular system was almost totally distorted.

M. incognita and *M. hapla* larvae penetrated within 48 h after inoculation. *M. incognita* larvae were cigar-shaped, and approximately 325 μm in length and 65 μm in diam 14 days after inoculation. *M. incognita* females became pear-shaped, approximately 380-400 μm in length and 150-275 μm in diam at the widest point after 20 days. *M. hapla* females were similar in size 20 days after inoculation.

TABLE 1. Number of *Meloidogyne graminis*, *M. incognita*, and *M. hapla* larvae which penetrated and developed in three cultivars of zoysia from an original inoculum of 200 ± 10 larvae

Species of nematode	Cultivar of zoysia	No. of nemas per plant[a]	
		Range	Average
M. graminis	*Z. japonica*	26-175	85.9
	Meyer	40-126	93.8
	Emerald	43-102	72.0
M. incognita	*Z. japonica*	22-89	38.8
	Meyer	19-90	50.6
	Emerald	21-65	43.4
M. hapla	*Z. japonica*	0-31	19.6
	Meyer	0-86	32.0
	Emerald	0-22	10.0

[a]Counts from eight plants made 7, 14, 33, and 38 days after inoculation.

M. incognita females reached maximum size 24 days after inoculation, with some females attaining a size of 500 × 275 μm. *M. hapla* attained a maximum size of 575 × 325 μm about 32 days after inoculation. No egg masses were observed on *M. incognita* females after 24 days, but eggs containing some coiled larvae were observed in egg masses 28 days after inoculation. Egg masses were approximately 450 × 300 μm in size, and contained 150-300 eggs/egg mass. At 32 days, *M. hapla* had deposited only a few eggs. After 36 days, egg masses were approximately 300 × 150 μm in size, and contained 75-150 eggs/egg mass.

Giant-cell formation and other host tissue reactions to infection by *M. incognita* (Fig. 1-E) and *M. hapla* (Fig. 1-F) were similar. Observations of serial sections indicated an average of 7-8 giant cells per *M. incognita* and *M. hapla* female. Giant cells induced by *M. incognita* contained as many as 150 nuclei per mature cell, whereas *M. hapla* giant cells contained 50-100 nuclei. Enlargement of tissue around the infection sites of *M. incognita* and *M. hapla* was caused by both hyperplasia and hypertrophy of tissue in the vascular system and cortex.

Hot water treatment of nematode-infected roots.—Heat treatment of *M. graminis*-infected zoysia plants for 10 min at temp of 40, 42, 44, and 46 C did not cause a decrease in number of larvae recovered. There was a slight decrease in larvae recovered after the 48 C treatment, and the 50 C treatment apparently killed all nematodes in the roots. Results of the second test in which one-degree temperature increments from 45-55 C were used, are shown in Table 2. At 45-47 C there was no decline in number of nematodes observed or recovered. There was a slight decrease in number at 49 C, but exposure of roots to temp of 50 C and above was lethal to all nematodes. In both tests, the leaves of plants treated with temp of 40-50 C became yellow. In the second test, the leaves regained their green color before the plants were checked for nematodes 2 mo after treatment. Plants

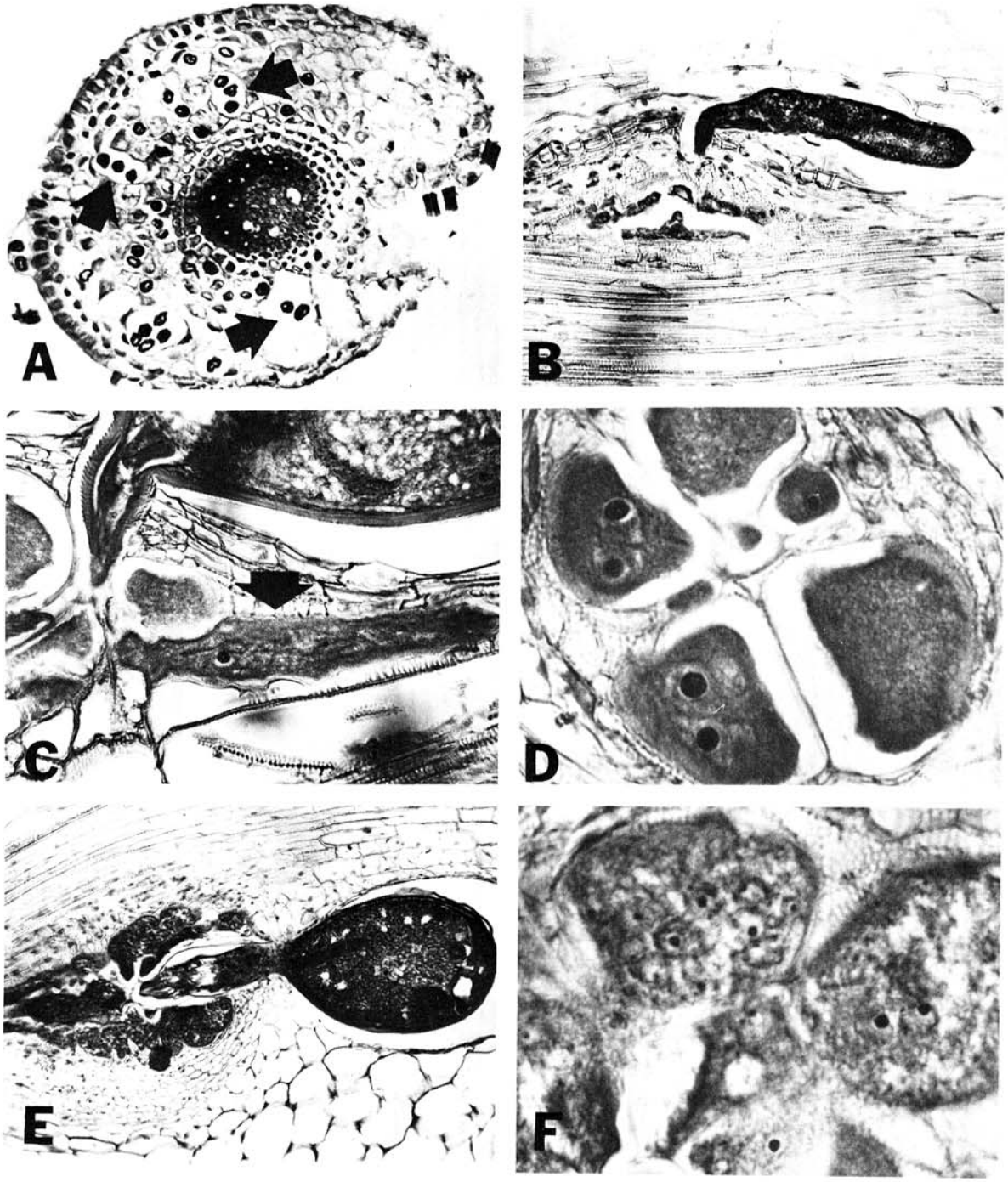

Fig. 1.-(A to F). A) Transverse section of infected Meyer zoysia root 48 h after inoculation showing *M. graminis* larvae (arrows) in cortex in groups; **B)** infected root 14 days after inoculation with *M. graminis* female anterior in vascular cylinder, and body in cortex; **C)** infected root 24 days after inoculation showing anterior end of female and elongated giant cell (arrow); **D)** giant cells in infected roots 24 days after inoculation showing homogenous granular protoplasm and enlarged nuclei and nucleoli; **E)** infected root 24 days after inoculation with *M. incognita* showing orientation of female and associated giant cells; **F)** giant cells stimulated by *M. hapla* 28 days after inoculation.

TABLE 2. Effect of 10 min hot water treatment on eradication of *Meloidogyne graminis* from infected Meyer zoysia roots

Treatment temp (C)	Number of larvae	
	Recovered from Baermann Funnels	Observed in indicator plants
Control	1,000 ± 100[a]	100 ± 10
45	1,000 ± 100	100 ± 10
46	1,000 ± 100	100 ± 10
47	1,000 ± 100	100 ± 10
48	500 ± 50	100 ± 10
49	500 ± 50	50 ± 10
50	0	0
51	0	0
52	0	0
53	0	0
54	0	0
55	0	0

[a]Counts for control and treatment temp of 45-47 C and 53-55 C represent the average from five plants; counts for treatment temp of 48-52 C represent the average from eight plants.

at 53, 54, and 55 C had varying amounts of necrotic leaf tissue and one plant treated at 54 C died.

DISCUSSION.—Emerald zoysia was reported to be a nonhost of *M. graminis* by Sledge (15), but in this study it was found to be very susceptible. Sunturf bermudagrass had not previously been reported as a host.

M. incognita had not previously been reported as a parasite of zoysia. It was a successful parasite of all three zoysia cultivars tested, and it could be a potentially important parasite of zoysia.

M. hapla was an inconsistent parasite of zoysia under the greenhouse conditions during these tests, and it developed slower than *M. graminis* and *M. incognita*. The slower development, however, possibly resulted from greenhouse temp which exceeded optimum temp for *M. hapla* development (2). *M. hapla* has been reported on bermudagrass (14), St. Augustinegrass, and Kentucky bluegrass (3), but it has not previously been reported infecting zoysia. Since *M. hapla* females reached maturity on zoysia under greenhouse conditions, it could become a pest of zoysia.

Hypertrophy of cells appeared to be the primary means of expansion of tissue surrounding *M. graminis* females. Hyperplastic tissues were much more evident around feeding sites of *M. incognita* and *M. hapla* females. This may account for the galling associated with *M. incognita* and *M. hapla* infections, and the only slight swelling associated with *M. graminis* infections.

In our tests, a temp of 28 C was most favorable for *M. graminis* development. Laughlin et al. (9) reported that *M. graminis* developed equally well at temp of 27 and 32 C.

Although a species name was not given by Heald and Wells (7) in their studies on the effect of hot water treatment of nematode-infected bermudagrass, the *Hypsoperine* sp. they used was probably *M. graminis*. They treated 1 × 3 in cores of sod, and reported that a hot water treatment of longer than 15 min at 50 C was necessary to eliminate the *Hypsoperine* sp. In our tests, a treatment of 10 min at 50 C was sufficient to kill all *M. graminis* nematodes. This difference can perhaps be attributed to the different grass species used, and the fact that the roots were washed free of sand in our tests. Both experiments indicate the feasibility of using hot water treatment to aid in the establishment of nematode-free turf.

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