Techniques for Determining Reproduction of *Meloidogyne graminis* on Zoysiagrass and Bermudagrass

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ABSTRACT

Murray, J. J., Poole, T. E., and Ostazeski, S. A. 1986. Techniques for determining reproduction of *Meloidogyne graminis* on zoysiagrass and bermudagrass. Plant Disease 70:559-560.

Two techniques were used to evaluate grass species and cultivars for resistance to the pseudo-root-knot nematode, *Meloidogyne graminis*, in greenhouse tests. After plants of eight species were inoculated with egg masses of *M. graminis*, the numbers of egg-laying females (ELF) on roots and the numbers of second-stage juveniles (J2) extracted from the soil around the roots were determined. Relative resistance ratings of genotypes based on the quickly derived ELF score on the roots were positively correlated with those based on numbers of juveniles produced on each genotype. More variation in susceptibility to *M. graminis* was found among bermudagrass (*Cynodon dactylon*) entries than among zoysiagrass (*Zoysia japonica*) entries tested. Some resistant bermudagrass genotypes were identified that may be useful on severely infested sites or as sources of germ plasm in a breeding program. None of the zoysiagrasses tested were resistant, but Emerald and Midwest were less susceptible than other genotypes. The possibility of success in breeding for resistance to *M. graminis* in both species is indicated by the amount of variability found among genotypes tested.

Of the nematodes recognized as turfgrass parasites, few are as widespread and potentially destructive as the pseudo-root-knot nematode, *Meloidogyne graminis* (Sledge & Golden) Whitehead (7). Since it was first described in 1962 (6), it has been associated with yellow, stunted grasses in the southeastern, southern, midwestern, and mid-Atlantic United States (1,2,4–6,8,9).

Previous studies (2,6,9) have demonstrated variability in susceptibility to *M. graminis* among turfgrass species and cultivars on the basis of juvenile numbers found in soil surrounding plant roots; however, no known source of resistance in any grass species has been identified. Nematode resistance is commonly defined as an inhibition or reduction of nematode reproduction (3,5). Symptom expression alone does not indicate nematode reproduction and may lead to erroneous values of resistance, as indicated by Fassuliotis et al (3) in snap bean (*Phaseolus vulgaris* (L.)). Variations reported in resistance among cultivars indicate that the development of *M. graminis*-resistant cultivars may be possible if a simple technique can be developed for screening and classifying genotypes.

We report the results from a comparison of two methods of evaluating *M. graminis* infections of turfgrasses: 1) determining the numbers of egg-laying females (ELF) on roots and 2) determining the numbers of second-stage juveniles (J2) extracted from soil around the grass roots. We used the ELF technique to determine the relative resistance to *M. graminis* among several cultivars and lines of zoysiagrass (*Zoysia japonica* Steud.) and bermudagrass (*Cynodon dactylon* (L.) Pers.).

MATERIALS AND METHODS

Species, entries, and cultivars of turfgrasses used in these experiments are listed in Tables 1–3. The pots in all three experiments were arranged in a split-plot design with entries as the whole-plot treatment and nematode-inoculated or noninoculated plants as the split-plot treatment. Experiment 1 had five replicates and experiments 2 and 3 had three replicates. Grasses were established by transplanting ten 10-day-old seedlings or 10 rooted cuttings in 15-cm-diameter plastic pots containing a mixture of sterilized sand and potting soil (1:1, v/v). Potting soil was a mixture of two parts chewacla silt loam soil (fine loamy mixed thermic fluvaquentic dystrochepts) and one part sterilized cow manure. Plants were inoculated with nematodes 4 wk after transplanting (experiments 1 and 2) or at transplanting (experiment 3).

Inoculum consisted of egg masses of *M. graminis* obtained from infected Tifgreen bermudagrass growing in flats of sand in the greenhouse. The original source of *M. graminis* for these experiments was obtained from infested Tufcote bermudagrass located in turfgrass field plots at the Beltsville Agricultural Research Center, Beltsville, MD. Infected plants were removed from the flat, and their roots were washed gently under running tap water. Egg masses were picked off the roots under magnification (×13) and temporarily stored in vials of distilled water. Plants were inoculated by pouring a vial of distilled water containing 10 egg masses into a depression in the soil at the center of each pot. Several sample hatchings in Baermann funnels containing distilled water showed that 10 egg masses yielded between 1,000 and 1,500 juveniles. Plants were maintained in the greenhouse at about 28 C with 14 hr of light (sunlight supplemented with incandescent light) per day and were fertilized twice with a solution of 20-20-20 (NPK) at the rate of 49 kg N/ha.

Plants in each experiment were evaluated 64–68 days after nematode inoculation. Soil was gently washed off the roots of inoculated plants. J2s from each pot in experiment 1 were extracted by pouring the soil suspension through a 325-mesh sieve and processing the sieve residue by the Baermann funnel technique. On the following day, J2s were removed from the funnel and counted under ×30. The washed roots were examined under ×13 and assigned an infection rating relative to the number of ELF present. The ELF score was based on a scale of 1–10, where 1 = no ELF, 2 = 1–5, 3 = 6–10, 4 = 11–15, 5 = 16–20, 6 = 21–25, 7 = 26–30, 8 = 31–35, 9 = 36–40, and 10 = more than 40 ELF. The roots from each pot in these experiments were assigned an ELF score.

In experiment 2, 16 turf-type bermudagrass cultivars, and in experiment 3, 11 zoysiagrass cultivars were evaluated for infection by *M. graminis* by the ELF technique.

RESULTS AND DISCUSSION

Uniform inoculations were obtained in all three experiments. Control plants showed no nematode infection and were therefore not included in analysis of data. Except for a small reduction in shoot growth rate of the most susceptible

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Accepted for publication 21 October 1985.

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Table 1. Relative resistance of several turfgrass species and cultivars to *Meloidogyne graminis* as determined by second-stage juvenile (J2) counts and the egg-laying female (ELF) rating system[w]

Cultivar and species	J2 (log_{10})	ELF rating[x]	Classification[y]
Meyer zoysia (*Zoysia japonica*)	3.5 a[z]	9.8 a	VS
Tufcote bermudagrass (*Cynodon dactylon*)	3.4 a	9.0 ab	VS
Tifgreen bermudagrass (*C. dactylon*)	3.1 a	8.6 b	VS
Kentucky 31 tall fescue (*Festuca arundinacea*)	3.1 a	5.8 c	S
Merion Kentucky bluegrass (*Poa pratensis*)	2.4 b	4.8 cd	S
Manhattan perennial ryegrass (*Lolium perenne*)	2.2 bc	3.8 d	S
Newport Kentucky bluegrass (*P. pratensis*)	1.9 c	3.8 d	S
Penncross creeping bentgrass (*Agrostis palustris*)	1.3 d	2.2 e	MR
Pennlawn red fescue (*F. rubra*)	0.5 e	1.0 f	R

[w] No control plots were nematode-infested and therefore were not included in analysis of the data.
[x] Rating scale of 1–10, where 1 = no ELF, 2 = 1–5, 3 = 6–10, 4 = 11–15, 5 = 16–20, 6 = 21–25, 7 = 26–30, 8 = 31–35, 9 = 36–40, and 10 = more than 40 ELF.
[y] VS = very susceptible, S = susceptible, MR = moderately resistant, and R = resistant.
[z] Means within a column followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 2. Relative ratings of turf-type bermudagrass for resistance to *Meloidogyne graminis* determined with the egg-laying female (ELF) rating system[w]

Entry	ELF rating[x]	Classification[y]
CC-15F	10.0 a[z]	VS
CCC-12F	10.0 a	VS
CCC-3F	10.0 a	VS
Belt-242	10.0 a	VS
Tufcote	9.7 a	VS
Belt-119	9.7 a	VS
CCC-10F	8.3 a	VS
CCC-4F	8.3 a	VS
CCC-5F	8.3 a	VS
E-29	6.0 b	S
St. Joseph	5.3 b	S
A-29	4.7 bc	S
Midiron	3.3 cd	MR
C-29	2.7 d	MR
Westwood	1.0 e	R
West Borough	1.0 e	R

[w] No control plots were nematode-infested and therefore were not included in analysis of the data.
[x] Rating scale of 1–10, where 1 = no ELF, 2 = 1–5, 3 = 6–10, 4 = 11–15, 5 = 16–20, 6 = 21–25, 7 = 26–30, 8 = 31–35, 9 = 36–40, and 10 = more than 40 ELF.
[y] VS = very susceptible, S = susceptible, MR = moderately resistant, and R = resistant.
[z] Means followed by the same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 3. Relative ratings of zoysiagrasses for resistance to *Meloidogyne graminis* resistance determined with the egg-laying female (ELF) rating system[w]

Entry	ELF rating[x]	Classification[y]
R-52-25	9.3 a[z]	VS
Meyer	9.3 a	VS
52-22(6)	9.3 a	VS
21-15(24)	9.0 a	VS
14-21(F)	9.0 a	VS
Zoysia tenuifolia	8.7 a	VS
Z. matrella L.	8.7 a	VS
Forbes	8.7 a	VS
41-21(3)	7.3 a	VS
Emerald	5.0 b	S
Midwest	4.7 b	S

[w] No control plots were nematode-infested and therefore were not included in analysis of the data.
[x] Rating scale of 1–10, where 1 = no ELF, 2 = 1–5, 3 = 6–10, 4 = 11–15, 5 = 16–20, 6 = 21–25, 7 = 26–30, 8 = 31–35, 9 = 36–40, and 10 = more than 40 ELF.
[y] VS = very susceptible and S = susceptible.
[z] Means followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

cultivars, visible plant symptoms of nematode infections were not observed during these studies.

In experiment 1, ELF ratings and J2 numbers were positively correlated (0.69, $P < 0.01$). A wider range of mean scores for entries was obtained with ELF ratings (1.0–9.8) than with the J2 numbers (0.5–3.5), indicating that the ELF rating may be more accurate than the J2 numbers for detecting small differences among genotypes (Table 1). Relative classification of the grasses for nematode resistance was obtained from both the ELF ratings and J2 numbers. These classifications are substantiated by results of previous studies of *M. graminis* with the same turfgrass species and cultivars (2,9).

These results indicate that the ELF rating can be used with the same degree of confidence as J2 numbers and will permit detection of smaller differences in resistance among genotypes. This method also requires considerably less time than that required for J2 counts because soil extraction of nematodes is not needed and egg masses are easier to count. The ELF technique may also be more easily standardized.

In experiment 2, two bermudagrass cultivars, Westwood and West Borough, were classified as resistant to *M. graminis* and two cultivars, C-29 and Midiron, were classified as moderately resistant (Table 2). Other cultivars were classified as susceptible. All cultivars showing resistance originated from a breeding program at Kansas State University. Midiron was the only commercially available cultivar tested that expressed a significant level of nematode resistance ($P < 0.05$).

The 11 zoysiagrasses tested in experiment 3 had ELF scores of 4.7 or higher (Table 3); thus all were classified as susceptible. However, Emerald and Midwest had significantly lower ELF scores than the other genotypes.

Inoculum of *M. graminis* used for experimental tests usually consisted of blended infected rootstock, infested soil, suspensions of J2, or egg masses. With the ELF germ plasm rating system, egg masses could be obtained from susceptible plants within a test for use as inoculum in an on-going screening program. Using egg masses would also have fewer limitations than J2 numbers when a relatively small, easily standardized quantity of inoculum is needed. Additional advantages of the ELF rating system include 1) continuous cultivation of inoculum, i.e., only the number of egg masses needed could be taken and source plants repotted; 2) availability of a reliable source of inoculum, and 3) a low chance of contamination by other parasitic nematodes or disease incitants.

We demonstrated that the ELF rating system is simpler and more reliable than determining J2 numbers for screening turfgrasses for *M. graminis* resistance and should be easily incorporated into a breeding program for development of resistant grass cultivars. The bermudagrasses Westwood and West Borough were resistant genotypes that may be useful germ plasm sources for developing resistant cultivars. Although none of the zoysiagrass genotypes tested were resistant, the significant differences obtained in levels of infection suggest that higher levels of resistance may be found by screening additional genotypes or breeding may increase the levels of resistance.

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