Interaction of Meloidogyne naasi, Pratylenchus penetrans, and Tylenchorhynchus agri on Creeping Bentgrass[1]

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Abstract: The pathogenicity and interactions of *Meloidogyne naasi, Pratylenchus penetrans*, and *Tylenchorhynchus agri* on 'Toronto C-15' creeping bentgrass, *Agrostis palustris*, was studied in a long-term greenhouse experiment. Based on dry weights of roots and clippings, *M. naasi* alone and in all combinations with *P. penetrans* and *T. agri* was highly pathogenic to creeping bentgrass. *P. penetrans* and *T. agri* alone and in combination inhibited root growth but adversely affected top growth only when the two were co-inoculated. In combination, the effects of each species on top growth were additive, with *M. naasi* the dominant pathogen. Creeping bentgrass was an excellent host for *M. naasi* and *T. agri*, but a poor host for *P. penetrans*. *T. agri* inhibited population increase of *M. naasi*, indicating nematode-nematode competition, but neither *T. agri* nor *P. penetrans* was affected by any of the combinations. *Key words: Agrostis palustris*, root-knot nematode, lesion nematode, stunt nematode, pathogenicity, population dynamics, nematode complexes.

In nature, cohabitation in the soil by different nematode genera and species as well as by other microorganisms occurs universally. Although studies involving interactions of nematodes with fungi, bacteria, and viruses have been relatively common, studies of nematode-nematode interactions have received less attention.

Nine genera of plant-parasitic nematodes have been found associated with creeping bentgrass, *Agrostis palustris* Huds., on Illinois golf course greens (8, 17). Within three of these genera, *Meloidogyne naasi* Franklin and *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven are parasitic on bentgrass (7, 14), and species of *Tylenchorhynchus* are considered largely responsible for turfgrass decline (16, 18). However, no previous reports demonstrated that these nematodes, singly or in combination, were actually detrimental to bentgrass.

The factors which influence the dynamics of nematode populations containing more than one species are essentially unknown. However, intergeneric inhibition of one plant-parasitic species by another is known to occur (1, 3, 5, 9, 11, 12, 15), and a synergistic interaction between genera has been recorded (2). Intrageneric dominance of one species over another has also been reported (13).

The objectives of this investigation were to determine the effects of *M. naasi, P. penetrans*, and *Tylenchorhynchus agri* Ferris, singly and in combination, on growth of creeping bentgrass and to determine the population dynamics of each nematode species.

MATERIALS AND METHODS

Nematodes used in this study were obtained from our greenhouse or laboratory stock cultures which originated from the following sources: *M. naasi*—creeping bentgrass, *A. palustris*, putting green, DuPage County, Ill.; *T. agri*—field of soybean, *Glycine max* (L.) Merr., Marion County, Ill.; *P. penetrans*—alfalfa, *Medicago sativa* L., callus tissue supplied by L. R. Krusberg, University of Maryland. Eggs of *M. naasi* were separated from infected barley, *Hordeum vulgare* L. 'Traill' and 'Trophy', by mincing chopped roots in a blender for 2 min, passing the blended material through 35- and 200-mesh screens, and collecting the eggs on a 325-mesh screen. Eggs were concentrated by centrifugation and stored in 0.3 M sodium chloride at 25 C; infective larvae were obtained using the modified Baermann extraction apparatus described by Amosu (1). *T. agri* was extracted from soil around roots of red clover, *Trifolium pratense* L. 'Kenland', using a modification of the Christie-Perry method (4). *P. penetrans* was mist-extracted from infected alfalfa callus tissue. Extracted nematodes were stored at 7 C and used within 4 days.

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Ninety-six clay pots, 15.5 cm diam, were filled with steam-pasteurized sandy loam soil. A 50-ml plastic beaker was depressed 5 cm deep into the center of each pot to create an inoculation site. Single-node stolon sections of 'Toronto C-15' creeping bentgrass were obtained from a stock culture propagated from a single stolon. Twelve sections were arranged around the beaker 1.5 cm outside its edge. The beaker was removed prior to inoculation.

Individual nematode species, alone and in all possible combinations, were added to the pots 4 weeks after planting. Treatments consisted of the following: 1) uninoculated control; 2) *M. naasi*; 3) *P. penetrans*; 4) *T. agri*; 5) *M. naasi* + *P. penetrans*; 6) *M. naasi* + *T. agri*; 7) *P. penetrans* + *T. agri*; 8) *M. naasi* + *P. penetrans* + *T. agri*. Inoculum in sterile distilled water was placed in the depression created by the removal of the beaker and the depression filled with soil. One-thousand nematodes of each species were used in all treatments but the control, which received sterile distilled water only. The experiment was divided into two 48-pot blocks, and each of the eight treatments within a block was replicated six times in a randomized complete block design on a greenhouse bench. A soluble fertilizer was added to all pots at approximately 2-week intervals. Greenhouse temperatures fluctuated between 20 C and 27 C, night and day, respectively.

All plants were trimmed to a height of 5 cm every 4 weeks, and the clippings were oven-dried and weighed. One block was removed 6 months, and the other 10 months, after inoculation. At the appropriate time, all above-ground plant parts were removed, and the soil was washed from the roots into 5 liters of water. The numbers of soil-inhabiting nematodes were determined after extracting them from 1 liter of the total soil-water suspension by a modification of the Christie-Perry method (4). Roots were blotted to remove excess water, cut into small sections, mixed and weighed. One-third of the root system then was placed in a mist chamber for 8 days and the extracted endoparasites were counted. Another third was stained with cotton-blue in lactophenol and minced in a blender for 2 min, and the *M. naasi* population determined. The final third was oven-dried for 4 days and weighed.

RESULTS

M. naasi, singly and in all combinations with *T. agri* and *P. penetrans*, significantly (P=0.01) reduced total clipping weight of 'Toronto C-15' creeping bentgrass (Table 1). *P. penetrans* and *T. agri* alone had no significant effect on clipping yields, but when these species were combined, a significant (P=0.05) reduction in top growth occurred. Where all three species were combined, clipping weight was significantly (P=0.05) lower than weights of all other treatments.

No significant differences in root weights were observed for the various treatments 6 months after inoculation (Table 1), but after 10 months, root weight in each inoculated treatment was significantly (P=0.01) lower than that of the control. Only in those treatments in which *M. naasi* was present, however, were root weights below those recorded at 6 months. Roots in treatments containing *M. naasi* were heavily galled at both 6 and 10 months.

Six months after inoculation, the population level of *M. naasi*, when alone, had increased 418 times and was significantly (P=0.01) higher than the population when combined with *T. agri* and/or *P. penetrans* (Table 2). At 10 months, the levels of *M. naasi* alone and in combination with *T. agri* were significantly (P=0.01) lower than those at 6 months. No significant differences in population levels occurred among the different combinations at 10 months.

Six months after inoculation, populations of

TABLE 1. Effects of *Meloidogyne naasi*, *Pratylenchus penetrans*, and *Tylenchorhynchus agri*, alone and in combination, on growth of 'Toronto C-15' creeping bentgrass.

Treatment[a]	Cumulative dry clipping weight (g)[b]	Dry root weight (g)[b]	
		6 months	10 months
Control	45[a]	2.3	7.0
M	38[cde]	2.5	1.8[bc]
P	42[abcd]	3.0	4.5[a]
T	44[ab]	2.3	3.4[abc]
M-P	39[bcde]	3.4	2.3[abc]
M-T	38[de]	2.3	2.1[bc]
P-T	41[abcd]*	3.1	3.9[ab]
M-P-T	34[e]**	2.0	1.4[c]

[a]M, P, and T = *M. naasi*, *P. penetrans*, and *T. agri*, respectively.

[b]Each figure is the mean of six replications. Values followed by the same letter do not differ (P=0.01) according to Duncan's new multiple range test.

*Significantly (P=0.05) different from the control.

**Significantly (P=0.05) different from all other treatments.

TABLE 2. Number of *Meloidogyne naasi*, *Pratylenchus penetrans*, and *Tylenchorhynchus agri* recovered from 'Toronto C-15' creeping bentgrass 6 and 10 months after inoculation alone and in combination.

Treatment[a]	Number of nematodes[b] 6 months	10 months
	M. naasi	
M (control)	417,531	142,801[c]
M-P	184,111*	135,433
M-T	194,690*	115,424[c]
M-P-T	177,550*	150,306
	P. penetrans	
P (control)	618	901
P-M	423	2200[c]*
P-T	301	526
P-M-T	540	1194
	T. agri	
T (control)	27,522	178,033[c]
T-M	32,297	211,541[c]
T-P	50,300	178,000[c]
T-M-P	39,000	184,250[c]

[a]M, P, and T = *M. naasi*, *P. penetrans*, and *T. agri*, respectively.
[b]Each figure is the mean of six replications. Initial inoculum level was 1000 of each species.
[c]Significantly (P=0.01) different from the population level at six months.
*Significantly (P=0.01) different from the control.

P. penetrans were approximately 50% lower than the initial inoculum level of 1000; numbers had not increased greatly even at 10 months (Table 2). Only where this species was combined with *M. naasi* was there a significant (P=0.01) increase in population over that at 6 months and over the control at 10 months.

When compared with the initial inoculum level, numbers of *T. agri* in all treatments had increased by a minimum of 28 times at 6 months and 178 times at 10 months (Table 2). Populations in all treatments at ten months were significantly (P=0.01) higher than they were at 6 months; however, no significant differences were found among the different combinations at either period.

DISCUSSION

M. naasi, alone and in all combinations with *T. agri* and *P. penetrans*, was highly pathogenic to 'Toronto C-15' creeping bentgrass. Although production of root abnormalities on this host by *M. naasi* has been shown previously (7), our results constitute the first evidence that this, or any other nematode, has the capability of retarding growth of creeping bentgrass. Previous experiments with *M. naasi* were less than 4 months in duration. Our study demonstrates that certain nematode species, after prolonged contact with bentgrass, may, in fact, be a major limiting factor in its production and maintenance. *P. penetrans* and *T. agri* alone and in combination reduced root growth, but to a lesser extent than did *M. naasi*. Top growth was adversely affected by *P. penetrans* and *T. agri* only when the two were combined, possibly because the plants were under very little temperature and moisture stress. These results indicate that when *M. naasi*, *P. penetrans*, and *T. agri* coexist on creeping bentgrass, the effects of each on top growth are additive, and that *M. naasi*, the dominant species, is responsible for the greatest amount of growth retardation.

Creeping bentgrass was an excellent host for *M. naasi* as well as for *T. agri*, both of which increased to high population levels. However, populations of *M. naasi* at 6 months were lower when in combination with *P. penetrans* and *T. agri* than when alone, indicating that nematode-nematode competition may have existed during the preceding period. Competition in varying degrees between two or more different nematodes species has been demonstrated by a number of other workers. Miller and Wihrheim (12) found that *Heterodera tabacum* Lownsbery and Lownsbery reduced infection and survival of *P. penetrans* and *Tylenchorhynchus claytoni* Allen on tobacco. *T. claytoni* suppressed *Trichodorus christiei* Allen on corn when co-inoculated (10). Feeding of *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven was reported as a major factor limiting populations of a *Meloidogyne* sp. on pineapple (6). Amosu (1) found that reproduction of *T. agri* and *P. penetrans* was retarded when each was in combination with *Meloidogyne hapla* Chitwood on red clover.

We conclude that the low populations of *M. naasi* when in combination with *P. penetrans* and *T. agri* were due to nematode-nematode interaction, whereby one species inhibited population increase of the other. The overall decrease in populations of *M. naasi* in all treatments from the sixth to the tenth month probably was due to the observed reductions in the root systems.

Agrostis palustris (=*A. stolonifera* L.) was considered susceptible to injury by *P. penetrans* in field tests conducted by Oostenbrink *et al.*

(14), who found populations of 1000-10,000/10 g of roots. The low levels of reproduction in our study may have been due to plant clonal differences. Because of the low reproduction, no conclusion could be drawn concerning the effect of *M. naasi* and *T. agri* populations on that of *P. penetrans*. The significant increase in the number of *P. penetrans* when it was combined with *M. naasi* may have resulted from the relatively extensive root system associated with this combination at 6 months.

T. agri populations at 10 months were higher than at 6 months, and were not significantly affected by the presence of *M. naasi* or *P. penetrans*. Because *T. agri* is a migratory ectoparasite feeding mainly on epidermal cells (1), growth retardation or destruction of roots would not be as limiting a factor in its reproduction as it would be with the sedentary endoparasite *M. naasi*. Consequently, the reduction in the overall root system after 6 months would not affect *T. agri* as much as *M. naasi*. Thus, the contrasting trends in the population dynamics of these two species between 6 and 10 months probably was caused by a differential availability of feeding sites.

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