Pathogenic Potential of Tylenchorhynchus dubius on Selected Turfgrass[1]

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Abstract: Tylenchorhynchus dubius was observed to feed on 'Toronto' creeping bentgrass and 'Merion' Kentucky bluegrass, and was a key participant in reducing the vegetative growth of both grass species. The severity of foliar and root macrosymptoms incited by *T. dubius* to 'Toronto' bentgrass was greater on plants grown at 16 C than on plants maintained at 21, 27 and 32 C. These parasitized plants exhibited a suppression of secondary stolon formation, shortened internodes and premature inflorescence initiation. Initial inoculum densities of 500 and 1000 nematodes/test pot produced similar pathogenic effects on the host. Feeding was primarily on root hairs and epidermal cells immediately behind the meristematic region. No necrotic lesions or other diagnostic symptoms were visible at the feeding sites. Nematodes were not observed inside the roots.
Key Words: stunt nematode, temperature, feeding.

INTRODUCTION

In 1954, Troll and Tarjan (9) found *Tylenchorhynchus* spp. to be the most widely distributed phytoparasitic nematode associated with turfgrasses in Rhode Island. They concluded that *T. claytoni* Steiner and *T. dubius* (Bütschli) Filipjev were likely to play major roles in devitalizing bentgrass (*Arostis palustris* Huds.) turf. Taylor et al. (7) recovered *Tylenchorhynchus* spp. from all bentgrass golf greens sampled in Illinois. Sumner (6) reported that *Tylenchorhynchus* spp. appeared to contribute to fading-out of bluegrass in *in vitro* studies. Troll and Rohde (8) reported that *T. claytoni* was pathogenic to creeping red fescue (*Festuca rubra* L.), did not effect Kentucky bluegrass (*Poa pratensis* L.) and appeared to stimulate shoot growth of annual ryegrass (*Lolium multiflorum* Lam.). A survey of poorly growing 'Toronto' creeping bentgrass (C-15) turf areas and 'Merion' Kentucky bluegrass lawns in Michigan showed the occurrence of *T. dubius* often in populations exceeding 500 nematodes/100 cc of soil (Laughlin and Vargas, unpublished data). The use of nonfumigant nematicides in Michigan resulted in lawns of a darker green color and more vigorous foliar growth than untreated areas within the same lawns (4). These treatments concurrently reduced the populations of *T. dubius* associated with 'Merion' Kentucky bluegrass turf. To investigate the pathogenic potential of *T. dubius* on 'Toronto' creeping bentgrass and 'Merion' Kentucky bluegrass, the following studies were initiated.

MATERIALS AND METHODS

Pathogenicity Studies. Five cuttings of 'Toronto' creeping bentgrass previously rooted in distilled water were placed in washed sand, which had been fumigated with methyl bromide, in a 7.6-cm diam plastic pot. Population densities of 500 and 1000 individuals of *T. dubius* were pipetted directly onto the roots. Supernatant, which was

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nematode-free but contained associated microorganisms, was applied to the roots of control plants in this and all preceding studies. All roots were covered with additional sand. Pots then were placed in washed sand in 20 X 35 cm plastic wash tubs and were submerged to within 2.5 cm of the top of the tubs in temperature-controlled water tanks maintained at 16, 21, 27 and 32±1 C. Four replicates of each inoculum density were placed in each of the four temperature tanks. Plants were watered as needed with distilled water, and 25 ml of a complete Hoagland's solution (1) was added at monthly intervals. Ninety days after initiation of the experiment, foliar parts were removed from all pots, roots were washed free of sand and both were dried for 48 hr at 100 C and weighed.

To investigate more closely the influence of *T. dubius* on foliar and root growth of 'Toronto' bentgrass plants, individual rooted tip cuttings were planted in 100-cc plastic tubes containing sand and infested with 200 nematodes. Non-infested control plants were prepared as previously described. Tubes were placed in sand-filled 12.7-cm-diam plastic pots and maintained at a soil temperature of 16±1 C in a water bath. After 30 days, top growth from six replicates was harvested and weighed, and stolon and internode lengths determined. Roots were removed from the tubes, washed free from soil and blotted dry to remove excess water, and fresh root weights were determined.

To investigate the pathogenic potential of *T. dubius* on 'Merion' Kentucky bluegrass, seeds were germinated in sterilized sand and grown until the seedlings reached a height of 2.5 cm. Ten plants were placed in 7.6-cm plastic pots containing a sand-loam-peat (1:1:1), steam pasteurized soil mixture. Approximately 300 *T. dubius* were added to the roots in each pot. Additional pots free of phytoparasitic nematodes, prepared as indicated above, served as controls. Pots were maintained at 16±1 C for the first 14 days and then at 21±1 C for the duration of the experiment.

Foliar fresh weights were determined at monthly intervals, and, after 90 days, roots were washed free of soil, excess water was removed and fresh weights were recorded. Nematodes were recovered from soil using a modification of the centrifugation-flotation technique (2).

Feeding Activity. To assess the feeding activity of *T. dubius*, transparent plastic petri dishes were partially filled with a 1.5% water agar. 'Merion' Kentucky bluegrass seedlings and rooted tip cuttings of 'Toronto' creeping bentgrass were placed on the agar and a nematode suspension was pipetted onto the surface of the roots. The plates were covered and incubated at 21 C. Periodically, the plates were placed under a stereoscopic microscope to observe nematode feeding activity.

RESULTS AND DISCUSSION

Oven-dry foliar weights of 'Toronto' creeping bentgrass were significantly reduced by *T. dubius* after 90 days at 16, 21 and 32 C but not at 27 C. Root weight was significantly reduced at all four temperatures (Tables 1 and 2). No differences in foliar or root weights were observed between inoculum densities of 500 and 1000 nematodes per pot. Macrosymptoms were most obvious at 16 C and included suppression of secondary stolon formation and a shortening of internode lengths (Table 3). These plants frequently exhibited inflorescence initiation, which may indicate a nematode induced physiological change in the plant toward early maturity.

T. dubius significantly reduced both foliar and root weights of inoculated 'Merion' Kentucky bluegrass (Table 4). Nematodes increased from an initial inoculum of 300 per pot to 1290 per pot over the 90-day period. The initial population was predominantly adults whereas the final population consisted of approximately 80% juveniles.

Direct observations of creeping bentgrass and Kentucky bluegrass roots showed *T. dubius* feeding primarily on root hairs and epidermal cells immediately behind the meristematic region of the roots. Penetrating with only the stylet the nematodes fed at a given location for not longer than 10 min, even when competition for feeding sites was minimal. No necrotic lesions or other diagnostic symptoms were visible at the feeding sites. Nematodes were not observed inside the roots. These observations support those of Klinkenberg (3) for *T. dubius* parasitizing *Poa annua*, and indicate that *T. dubius* is capable of inciting foliar and root reductions in both 'Toronto' bentgrass and 'Merion' Kentucky bluegrass.

Thus, according to the pathogenicity concept of Lownsbery and Thomason (5), *T. dubius* is a key participant in reducing vegetative growth of both 'Toronto' creeping

TABLE 1. Influence of inoculum density of *Tylenchorhynchus dubius* and temperature on oven-dry foliar weights of 'Toronto' creeping bentgrass over a 90-day period.

Inoculum density	Foliar weights (g) 16 C	21 C	27 C	32 C
0	5.42 a[a,b]	6.22 a	3.19 a	0.81 a
500	1.52 b	4.47 b	3.44 a	0.79 a
1000	1.60 b	4.92 b	3.35 a	0.57 b

[a]Each value is a mean of four replications.

[b]Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test. Comparisons are valid only within a given temperature.

TABLE 2. Influence of inoculum density of *Tylenchorhynchus dubius* and temperature on the oven-dry root weight of 'Toronto' creeping bentgrass over a 90-day period.

Inoculum density	Root weights (g) 16 C	21 C	27 C	32 C
0	4.62 a[a,b]	2.78 a	2.64 a	1.45 a
500	1.82 b	1.73 b	1.82 b	0.65 b
1000	1.82 b	1.82 b	1.65 b	0.80 b

[a]Each value is a mean of four replications.

[b]Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test. Comparisons are valid only with a given temperature.

TABLE 3. Influence of *Tylenchorhynchus dubius*[a] on individual stolon growth of 'Toronto' creeping bentgrass over a 30-day period.

Treatment	Stolon lengths (cm) Primary	Secondary	Internode length (cm)	Fresh weights (g) Foliar	Root
Inoculated	15.83[b]	0.83	1.87	.01	.14
Noninoculated control	41.67**	79.67**	3.85**	.11**	.62**

**Student-t value significant at 1% level.

[a]Initial inoculum density was 200 T. dubius/100 cc of soil.

[b]Each value is a mean of six replications.

TABLE 4. Influence of *Tylenchorhynchus dubius*[b] on the fresh foliar and root weights of 'Merion' Kentucky bluegrass over a 90-day period.

	Harvest periods (Months after treatment)				
	Fresh Weight (g)				Nematodes
	Foliar			Root	In Soil
Treatment	1	2	3	3	(No./pot)
Inoculated	0.35[a]	0.26	0.25	0.36	1290[b]
Noninoculated control	0.59*	0.48*	0.39*	1.11**	0

[a]Each value is a mean of four replications.

[b]Initial inoculum density was 300 T. dubius/pot.

*Student-t value significant at 5% level.

**Student-t value significant at 1% level.

bentgrass and 'Merion' Kentucky bluegrass. The severity of growth reduction incided by *T. dubius* to 'Toronto' bentgrass was influenced by soil temperature. These greenhouse data may explain field observations in Michigan. Turf growth of 'Toronto' bentgrass in these areas was poorest during early spring and late fall months, when soil temperatures were below 20 C. Symptoms were minimized during midsummer when soil temperatures were above 25 C.

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