# *Meloidogyne microtyla*: Pathogenicity to Orchard Cover Grasses, Survival in Stored Soil, and Reproductivity After Storage

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**ABSTRACT**

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*Meloidogyne microtyla* was most destructive on creeping red fescue, reducing accumulated top clippings 42% over 18 wk. Kentucky bluegrass was not affected by this root-knot nematode. The greatest number of juveniles developed on the roots of perennial ryegrass, and the lowest number, on creeping red fescue. Numbers of juveniles recovered from soil stored at 5 C increased in a cyclical manner over a 1-yr period, whereas those from soil stored at 22 C diminished to almost undetectable numbers within 98 days. The occurrence of diapause in *M. microtyla* is proposed. Reproductivity of *M. microtyla* on tomato in soil previously stored at 5 C was nine times that of *M. microtyla* in soil from fresh tomato + grass cultures and 100 times that of *M. microtyla* in soil stored at 22 C.

Particular turfgrasses are used as orchard covers in Ontario because of their suppression of the root-lesion nematode *Pratylenchus penetrans* Cobb (5). Recently, the root-knot nematode *Meloidogyne microtyla* Mulvey et al, described in 1975 (2), was found on creeping red fescue (*Festuca rubra* L.) in an apple orchard in the Georgian Bay area. The cover grass was severely damaged and overrun by dicotyledonous weeds (3). A recent study by the authors showed that *M. microtyla* reproduced on both monocotyledons and dicotyledons, but Gramineae were the preferred hosts (6). Because of the importance of grasses in the management of orchards in Ontario, the pathogenicity of *M. microtyla* to grasses, survival in stored soil, and reproductivity after storage were studied.

## MATERIALS AND METHODS

The population of *M. microtyla* originated from creeping bentgrass (*Agrostis stolonifera* L.) from a golf green at Delhi, Ontario. The nematode was cultured in a greenhouse in 12-cm clay pots filled with Fox loamy sand planted to both tomato (*Lycopersicon esculentum* Mill. 'Stakeless') and creeping red fescue.

**Growth responses of grasses to *M. microtyla*.** The pathogenicity of *M. microtyla* was determined on four turfgrasses commonly used as orchard cover grasses because of their suppressive effect on *P. penetrans*. Fox loamy sand infested with *M. microtyla* was mixed with greenhouse potting soil (Vineland silt loam, Fox loamy sand, and peat [2:1:1, v/v/v]) to provide 15 juveniles per gram of soil in a 17.5-cm clay pot. Creeping red fescue (*F. rubra* 'K8-149'), Kentucky bluegrass (*Poa pratensis* L. 'Park'), perennial ryegrass (*Lolium perenne* L. 'Norlea'), and tall fescue (*F. arundinacea* Schreb. 'Kentucky 31') were seeded at 0.4, 0.35, 0.5, and 0.8 g per pot, respectively. Controls were prepared similarly with noninfested soil. The pots were plunged into nematode-free soil in a greenhouse groundbed, and moisture was maintained with an automatic watering system. The pots were arranged in a randomized block design with seven replicates. Grasses were clipped to 6 cm high and weighed at 2-wk intervals for 18 wk. The weights of the remaining tops and roots were determined at the end of the experiment. Juveniles were extracted from 50-g soil subsamples by the pan method (4) and from the roots by mist extraction, both for 2 wk.

**Effect of temperature on longevity of *M. microtyla* in stored soil.** Survival of *M. microtyla* in soil was studied under two storage temperature regimes, 22 C (warm-stored) and 5 C (cold-stored). Infested soil from tomato + grass cultures was passed five times through an automatic sieve-shaker to remove root debris and to obtain a uniform mix of eggs and juveniles. The prepared soil was divided into two 25-kg lots, placed in separate plastic bags, and loosely tied to retain moisture. One lot was stored at each of the given temperatures. Sampling began at day zero with subsequent samplings at 2-wk intervals for the first month and then approximately at monthly intervals for 1 yr. Eight samples were taken from each bag with a 2.5-cm-diameter soil core sampler. Juveniles were extracted from eight 50-g subsamples per temperature by the pan method (4) for 2 wk and counted.

**Reproductivity of *M. microtyla* after storage.** Reproductivity of *M. microtyla* was determined using the two soils from the preceding experiment after storage for 1 yr. The infested soil stored at 5 C was mixed with sterile soil to reduce the number of *M. microtyla* to the same level as that in the soil stored at 22 C. The soil previously stored at 22 C was held at 5 C during this preparation to prevent further decline in the *M. microtyla* population; the remainder of the soil from this treatment was retained at 5 C for 270 days. At the same time, unstored infested soil from tomato + grass cultures was also adjusted to the same population level as in the soil stored at 22 C to serve as a control. Eight 12.5-cm clay pots were filled with 990 g of each infested soil, and a single Stakeless tomato seedling was planted in each pot. The pots were arranged in a randomized block design with eight replicates on a greenhouse bench. After 17 wk, roots from each pot were examined for galls and placed in a mist chamber for 2 wk to extract

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juveniles. Juveniles were extracted from 50-g soil subsamples from each pot as before. Data were analyzed by analysis of variance, and least significant differences (LSD) were calculated.

## RESULTS

**Growth responses of grasses to *M. microtyla*.** The accumulated weight of clippings from *M. microtyla*-infected creeping red fescue, tall fescue, and perennial ryegrass was reduced after 18 wk by 42, 34, and 26%, respectively (Fig. 1). The 8% reduction in the weight of accumulated clippings of infected Kentucky bluegrass was not significant. Reduction in growth of the three affected grasses was evident after 6 wk (Fig. 1).

After the final clipping, only the fresh top weights of the infected creeping red fescue and perennial ryegrass were less than those of their respective controls (Table 1). The fresh weights of infected roots of all grasses were less than those of the controls (Table 1). The roots of perennial ryegrass supported the greatest number of juveniles (Table 2). The soil from the four infected grasses contained six or seven juveniles per 50 g.

**Effect of temperature on longevity of *M. microtyla* in stored soil.** In the survival study of *M. microtyla*, the number of juveniles in the soil stored at 22 C declined from about 5,000 to 250 per 50 g of soil after 56 days and thereafter declined to about 25 per 50 g of soil after 98 days (Fig. 2). In contrast, the number of juveniles in the soil stored at 5 C increased in a cyclical manner from 5,000 to almost 10,000 per 50 g of soil after 254 days (Fig. 2). The population progressed through four cycles during this period, each cycle peaking at a higher level than the previous one.

**Reproductivity of *M. microtyla* after extended storage.** Galls were found only on the roots of tomato planted in soil that had been stored at 5 C and on those planted in fresh, infested soil. At harvest, different numbers of juveniles per gram of root were recovered: 340 from tomato grown in soil stored at 5 C, 40 from tomato grown in fresh, infested soil, and 3 from tomato grown in soil stored at 22 C; $LSD_{5\%} = 35$.

The soil stored at 22 C after 98 days contained about 25 juveniles per 50 g. This soil was kept at 5 C for 2–3 wk while soil previously stored at 5 C and fresh-infested soil was diluted with sterile soil to the same population as the soil stored at 22 C. Samples taken from each of the three soils at the installation of the experiment in the greenhouse showed that the population had increased from 25 to 180 per 50 g in the soil previously stored at 22 C while that in the other two soils remained at 25–30 per 50 g of soil. After the soil previously stored at 22 C for 1 yr had been in cold storage (5 C) for about 200 days, the population increased to 770 juveniles per 50 g of soil, then declined to 340 after 270 days.

## DISCUSSION

Creeping red fescue should not be recommended as an orchard cover in the Georgian Bay fruit-growing area where *M. microtyla* is found. This grass was the most severely damaged of the four grasses tested, although it supported the smallest *M. microtyla* population. Kentucky bluegrass was least affected and is now our recommended cover grass for orchards infested with *M. microtyla*. Perennial ryegrass is affected only moderately by *M. microtyla*, but it cannot be recommended because it is conducive to a rapid population development of the nematode. However, the four grasses remain useful as cover crops for the suppression of *P. penetrans* where *M. microtyla* does not occur.

Although the number of juveniles in the soil stored at 5 C went through cycles, the increases could not have resulted

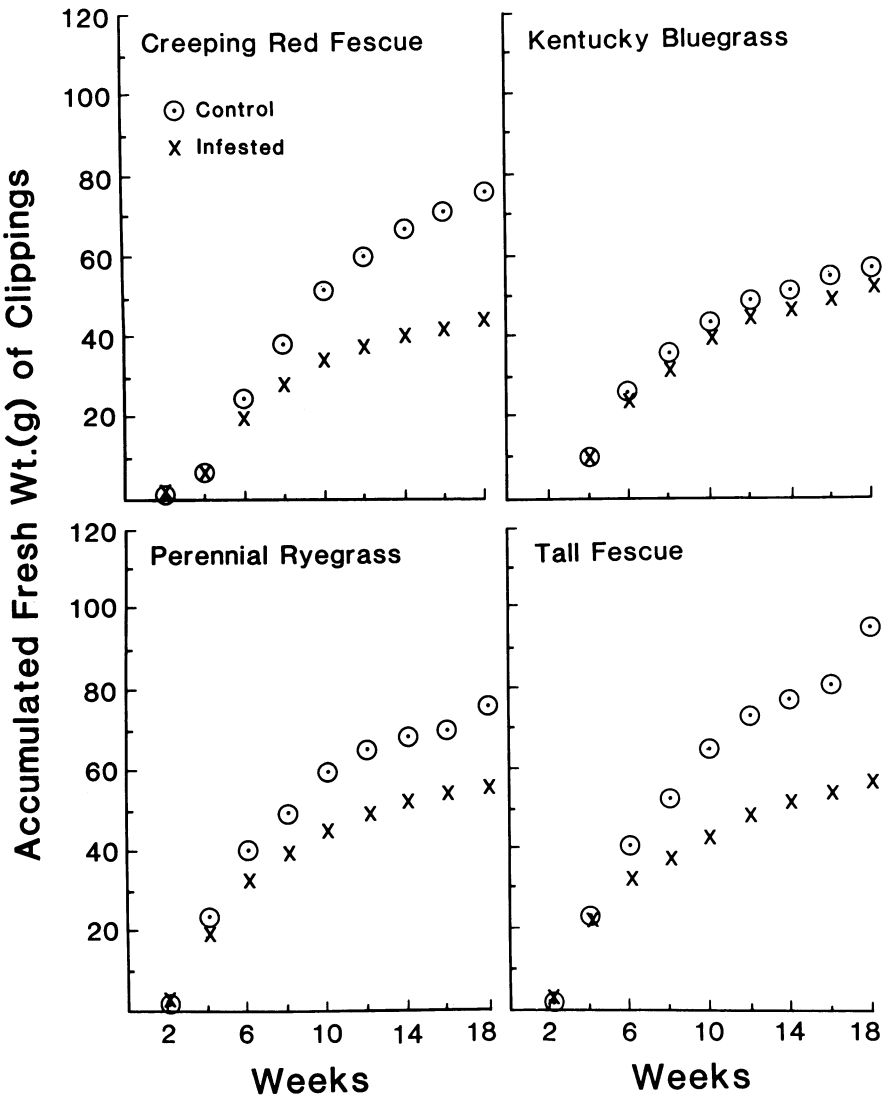


**Fig. 1.** Accumulated clippings from four orchard cover grasses grown in noninfested soil and soil infested with *Meloidogyne microtyla*. LSDs from accumulated clippings at 18 wk are creeping red fescue 13, Kentucky bluegrass ns, perennial ryegrass 9, and tall fescue 11 at 5%.

**Table 1.** Fresh weight (g) of remaining tops and roots of four grasses infected with *Meloidogyne microtyla* after the final clipping (18 wk)

| | Top | | | Root | | |
|---|---|---|---|---|---|---|
| Grass | Control | Infected | $LSD_{5\%}$ | Control | Infected | $LSD_{5\%}$ |
| Creeping red fescue (*Festuca rubra*) | 10.9 | 6.8 | 1.6 | 52.9 | 26.7 | 11.3 |
| Kentucky bluegrass (*Poa pratensis*) | 16.1 | 14.1 | ns | 100.3 | 70.3 | 18.5 |
| Perennial ryegrass (*Lolium perenne*) | 14.7 | 11.0 | 1.6 | 115.6 | 58.4 | 14.3 |
| Tall fescue (*F. arundinacea*) | 15.7 | 13.9 | ns | 55.6 | 43.0 | 8.2 |

**Table 2.** Average number of juveniles of *Meloidogyne microtyla* on four grasses 18 wk after inoculation

| Grass | No. of juveniles | |
|---|---|---|
| | Per root system | Per gram of root |
| Creeping red fescue (*Festuca rubra*) | 2,570 | 100 |
| Kentucky bluegrass (*Poa pratensis*) | 3,850 | 60 |
| Perennial ryegrass (*Lolium perenne*) | 46,200 | 790 |
| Tall fescue (*F. arundinacea*) | 6,900 | 160 |
| $LSD_{5\%}$ | 5,260 | 70 |

from reproduction. The population changes might be explained by diapause in the egg population. In culture before cold storage, each successive generation probably produced a larger number of eggs, a portion of which entered diapause. Subsequently, when diapause was broken in cold storage, the succession of generations resulted in the higher number of juveniles recovered with each cycle. Low-temperature breaking of diapause in egg masses of sedentary nematodes occurs in *Heterodera avenae* (1). This concept was further supported since in the soil stored at 22 C that was briefly chilled, a larger number of juveniles were recovered than before the soil was chilled. These juveniles probably emerged from surviving diapaused eggs; diapause was broken by chilling at 5 C. However, the occurrence of diapause as a winter-survival mechanism in other temperate-zone root-knot nematode species needs to be shown.

Reproductivity of *M. microtyla* in soil previously stored at 5 C was enhanced compared with that of *M. microtyla* in soil from fresh tomato + grass cultures or *M. microtyla* in soil stored at 22 C. Existing juveniles and those emerging from diapaused eggs in soil that had been stored at 5 C retained their infectivity probably because little body fat was used to sustain life, whereas those in soil stored at 22 C even though chilled for 2 wk probably used their body fat to such an extent that very few juveniles were infective (7). Juveniles in fresh soil were probably a mixture of infective and noninfective. This concept is compatible with previous research on the effects of starvation on the infectivity of *M. javanica* (7).


**Fig. 2.** Number of *Meloidogyne microtyla* juveniles recovered from infested soils stored at 22 and 5 C for 1 yr.

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