Nematode Reproduction on Endophyte-Infected and Endophyte-Free Tall Fescue

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ABSTRACT

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Infection of tall fescue (*Festuca arundinacea*) with the endophytic fungus *Acremonium coenophialum* has been shown to reduce nematode populations in field soils. We evaluated reproduction of three plant-parasitic nematodes on endophyte-infected (E+) and endophyte-free (E−) tall fescue in greenhouse tests. E+ plants had lower numbers of *Pratylenchus scribneri,* a migratory endoparasite, than E− plants, and roots of E+ plants had fewer egg masses and eggs of *Meloidogyne marylandi,* a sedentary endoparasite. However, levels of *Helicotylenchus pseudorobustus,* an ectoparasitic nematode, were not significantly different in pots of E+ and E− tall fescue. Reproduction of an undescribed *Meloidogyne* sp. on white clover was not affected by the presence of E+ or E− fescue.

Additional keyword: Poaceae

Tall fescue, *Festuca arundinacea* Schreb., is one of the most important forage crops in the southeastern United States and is grown on more than 1.4 million ha in Tennessee (16). Tall fescue is resistant to drought and tolerates a wide range of soil and climate conditions.

More than 80% of the tall fescue in Tennessee is infected with *Acremonium coenophialum* Morgan-Jones & W. Gams (16), a symbiotic endophytic fungus that grows intercellularly in the leaf sheath (2,10). Presence of the endophyte in tall fescue pastures is correlated with reduced weight gain in beef cattle, reproductive problems in mares, and other physiological symptoms in livestock that are referred to collectively as fescue toxicosis (2,22,27).

Fescue toxicosis results in annual economic losses of millions of dollars (27) and has prompted renovation of pastures with endophyte-free (E−) tall fescue seed. E− tall fescue is more difficult to establish and maintain than endophyte-infected (E+) tall fescue and is less resistant to heavy grazing (22) and drought (1,28).

The greater stress tolerance in E+ tall fescue compared to E− tall fescue may be the result in part of greater pest resistance. The presence of *A. coenophialum* in tall fescue deters feeding by aphids (*Rhopalosiphum padi* L. and *Schizaphis graminum* (Rondani)) (14), Argentine stem weevil (*Listronotus bonariensis* (Kuschel) (21), and larvae of fall armyworm (*Spodoptera frugiperda* (J. E. Smith)) (9). Populations of two plant-parasitic nematodes, *Pratylenchus scribneri* Steiner and *Tylenchorhynchus acutus* Allen, were lower in field plots of E+ tall fescue than in E− plots (28). And in greenhouse tests, fewer *Paratrichodorus minor* (Colbran) Siddiqi and *Helicotylenchus dihystera* (Cobb) Sher were associated with E+ tall fescue than with E− fescue (20).

The objectives of this study were to determine reproduction rates of *P. scribneri, H. pseudorobustus* (Steiner) Golden, and *Meloidogyne marylandi* Jepson & Golden in Jepson (13) on roots of E+ and E− tall fescue in the greenhouse and to elucidate the effects of E+ tall fescue roots in the rhizosphere of white clover (*Trifolium repens* L.) on reproduction of an undescribed species of *Meloidogyne* parasitic on clover. In an earlier report (15), *M. marylandi* was called *M. graminis* (Sledge & Golden) Whitehead.

P. scribneri and *H. pseudorobustus* have been shown to be parasitic on tall fescue (6,12,16,17). *P. scribneri* is an obligate migratory endoparasite that can move freely within roots and in the rhizosphere. This nematode has a wide host range and has been shown to restrict forage yield of tall fescue (17,19). *H. pseudorobustus,* an ectoparasite that lives in the rhizosphere, sometimes feeds with its body partially embedded in root tissue. Although considered a weak pathogen, it may incite root necrosis through interactions with other nematodes or pathogens. Such interactions may contribute to yield reduction (6). *M. marylandi* is known to parasitize both bermudagrass (*Cynodon dactylon* (L.) Pers.) and zoysia (*Zoysia japonica* Steudel) but has not been demonstrated to parasitize tall fescue. *M. graminis,* which has often been confused with *M. marylandi* (8), has been reported to be parasitic on tall fescue (13).

MATERIALS AND METHODS

Plants. Plants of tall fescue cultivar Kentucky 31 were grown from seeds obtained from one seed lot. Single seeds were planted in single peat pots in Promix (Premier Brands, Inc., Stamford, CT). After 1 mo, one tiller from each plant was tested for the presence of *A. coenophialum* with an indirect protein-A sandwich enzyme-linked immunosorbent assay (PAS-ELISA) (24). This procedure was repeated three times, and only plants with three consistent evaluations (E+ or E−) were used. Plants were divided into sets of three to four tillers, and tillers were transplanted into 500 cm^3 of steam-sterilized sand-soil (1:1) mix in clay pots 10 cm in diameter.

After infestation with suspensions of nematodes or nematode eggs, pots in each experiment were arranged in a randomized complete block design in the greenhouse. When fescue plants were harvested for evaluation, one tiller from each plant was again tested for the presence of *A. coenophialum.* The results of these tests were consistent with those of the original assays. Each experiment was repeated.

Host studies. *P. scribneri* in corn (*Zea mays* L.) root explant culture was obtained from R. N. Huettel (Plant Protection Institute, USDA-ARS, Beltsville, MD). This nematode was increased either on callus tissue of tall fescue cultivar Forager grown in Schenk and Hildebrandt basal medium (25) supplemented with 30 μM dicamba (3,6-dichloro-O-anisic acid, Velsicol Chemical Corporation), 30 g/L sucrose, 9 g/L Difco Bacto agar, and 1 g/L inositol, adjusted to pH 5.6 (5), or on Seneca Chief corn root explants grown in Gamborg's B-5 basal medium (Gibco Laboratories) with 10 g/L Difco Bacto agar adjusted to pH 5.7 (7,23).

Nematodes for inoculation were collected in a modified Seinhorst mist apparatus (26). A fine mist was sprayed over the callus for 10 sec every 10 min for 2 wk. Nematodes emerging from the callus were gathered at 24-hr intervals.

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Soil mix was infested with an aqueous suspension containing 1,200 (experiment 1) or 2,000 (experiment 2) nematodes per pot. Two milliliters of inoculum was pipetted into each of five 1-cm^3 holes spaced evenly around E− or E+ tall fescue plants, 3 cm from the tillers. Treatments in each experiment were replicated six times.

After 15 wk, plants were removed from the pots. Roots were washed, cut into 1-cm pieces, and macerated in a Waring Blendor with 100 ml of water for three 10-sec intervals at high speed (approximately 12,600 rpm). Nematodes were extracted from this suspension by a sugar flotation-centrifugation method (12). The same method was used to extract nematodes from a 100-cm^3 subsample of soil from each pot.

H. pseudorobustus was collected from a fescue pasture at Ames Plantation in southwestern Tennessee and maintained on sunflower (*Helianthus annuus* L.) in the greenhouse. Nematodes were extracted from stock cultures by the sugar flotation-centrifugation method. A 10-ml aqueous suspension containing 1,000 nematodes was pipetted into five 1-cm^3 holes in sand-soil mix in pots as described above. Treatments in each experiment were replicated eight times. After 8 wk in the first experiment and 10 wk in the second experiment, nematodes were extracted from a 100-cm^3 soil subsample from each pot (12).

M. marylandi was collected from bermudagrass turf in Knoxville, TN, and was maintained on bermudagrass in the greenhouse. Eggs were obtained by shaking root pieces in a 1% NaOCl solution (11) and sieving. Eggs were counted at ×40 magnification. A 10-ml suspension containing 3,400 (experiment 1) or 5,000 (experiment 2) eggs was poured onto 250 cm^3 of sand-soil mixture in clay pots 7 cm in diameter, E+ or E− tillers were planted, and pots were filled with the sand-soil mixture. There were eight replicates in experiment 1 and five in experiment 2. After 2 mo, roots were removed from the soil. Egg masses were stained by soaking roots 15 min in a solution of 0.15% aqueous phloxine B (30) and were counted at ×10 magnification. Roots were then cut into 1-cm pieces, and eggs were extracted in a 1% NaOCl solution.

Nonhost study. An undescribed species of *Meloidogyne* parasitic on white clover (3) was collected from a tall fescue-white clover pasture at Ames Plantation and maintained in the greenhouse on white clover. In all experiments, eggs were extracted by macerating roots in a Waring Blendor for three 10-sec intervals at high speed. Eggs were gathered on a sieve with 15-μm pores and were diluted with tap water to a concentration of 80 eggs per milliliter.

Preliminary experiments were conducted to determine the ability of this nematode to infect fescue. A 10-ml aliquot of the egg suspension was pipetted into each of five 1-cm^3 holes spaced evenly around tillers of E+ or E− fescue grown in clay pots 7 cm in diameter. After 8 wk, root systems were harvested, washed, and examined for galling. No galls were observed.

Ten E+ (treatment 1) or E− (treatment 2) tillers were transplanted around the periphery of plastic pots 20 cm in diameter. White clover was seeded in the center of the pots. White clover and tall fescue were allowed to grow together for 1 mo before inoculum was added. In treatment 3, only white clover was planted. A 10-ml aliquot of an aqueous egg suspension (80 eggs per milliliter) was pipetted into each of five 1-cm^3 holes placed randomly in the rhizosphere of the white clover in each pot. Total inoculum was 4,000 eggs per pot. Treatments were replicated eight times in the first experiment and five times in the second experiment.

After 10 (experiment 1) or 14 (experiment 2) weeks, white clover roots were harvested, washed, and weighed. Galls were counted on about one-third of the root system at ×10 magnification, and the number of galls per gram of root was calculated. This value was used to estimate total galls per pot. The entire root system was then cut into 1-cm pieces and

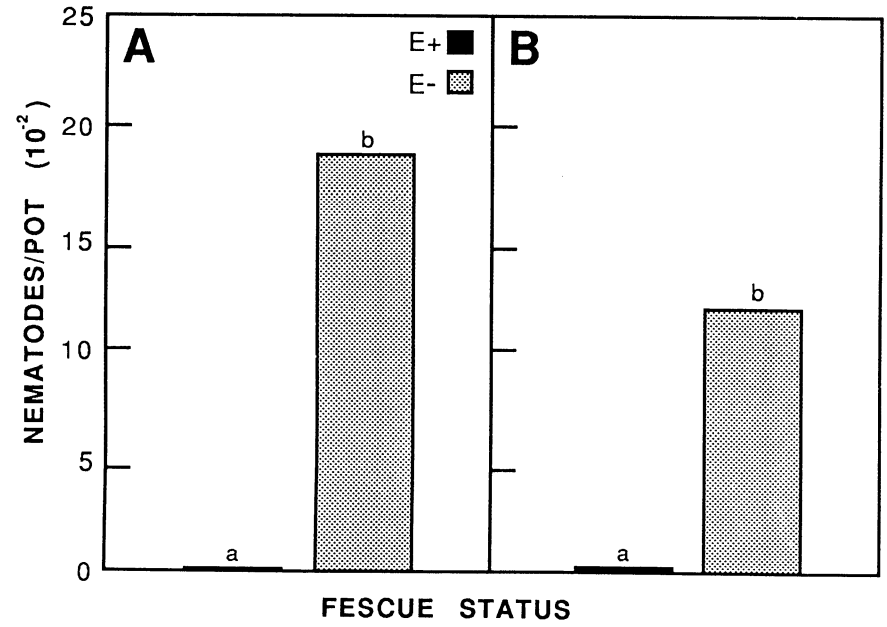

Fig. 1. Mean numbers of *Pratylenchus scribneri* on endophyte-infected (E+) and endophyte-free (E−) tall fescue. **A,** experiment 1; **B,** experiment 2. Columns headed by different letters are different at $P = 0.05$ (using an independent t test).

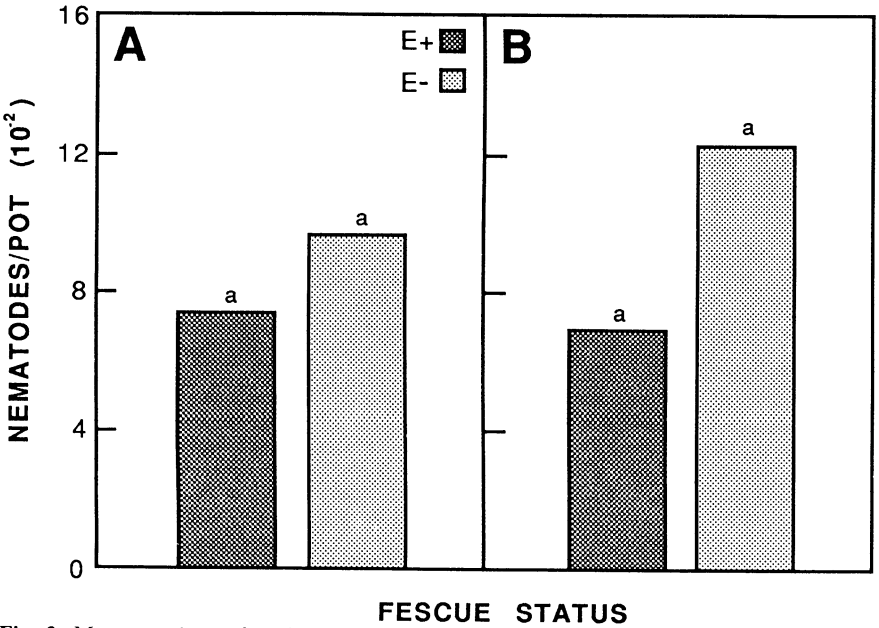

Fig. 2. Mean numbers of *Helicotylenchus pseudorobustus* on endophyte-infected (E+) and endophyte-free (E−) tall fescue. **A,** experiment 1; **B,** experiment 2. Columns headed by the same letter do not differ at $P = 0.05$ (using an independent t test).

macerated, and eggs were collected and counted as described above.

Statistics. Data were tested for significant differences among treatments with an independent *t* test for experiments with *P. scribneri, H. pseudorobustus,* and *M. marylandi,* and analysis of variance was done for experiments with *Meloidogyne* sp. For *Meloidogyne* sp., Duncan's new multiple range test was used for separation of means. In experiment 2 with *M. graminis,* data were normalized with a $\log_{10}(x+1)$ transformation before analysis of variance.

RESULTS

Host studies. At the conclusion of the experiments, numbers of *P. scribneri* and *H. pseudorobustus* on E− tall fescue were at or near initial inoculum levels; however, high numbers of juveniles observed in the final populations indicated that reproduction was occurring. Reproduction of *M. marylandi* was evidenced by the production of egg masses on roots but was less than that obtained routinely on bermudagrass.

Populations of *P. scribneri* were reduced severely on E+ fescue (Fig. 1). In experiment 1, no nematodes were isolated from E+ plants; in experiment 2, a total of eight nematodes were isolated from all E+ plants. Numbers on E− plants were at or below inoculum levels. In both experiments, final numbers of nematodes per replicate were significantly lower on E+ plants than on E− plants.

Final numbers of *H. pseudorobustus* on E+ tall fescue were slightly lower but not significantly different from those on E− tall fescue (Fig. 2). The number of nematodes varied greatly in both experiments (144–4,400 nematodes in E− plants and 280–1,140 nematodes in E+ plants).

Reproduction of *M. marylandi* was reduced on E+ tall fescue compared to E− fescue. Significantly fewer egg masses were associated with the roots of E+ fescue (Fig. 3A,B), and this was reflected in egg numbers (Fig. 3C,D). Egg masses on E+ fescue roots were also smaller and contained fewer eggs than those on E− fescue.

Nonhost study. The undescribed *Meloidogyne* sp. did not reproduce on tall fescue but reproduced abundantly on white clover. Reproduction of this nematode was not influenced by the endophyte status of the tall fescue grown with the clover (Fig. 4). Numbers of nematodes were significantly higher in pots where clover was grown without tall fescue than in pots containing either E+ or E− tall fescue; clover developed larger root mass in pots that did not contain fescue.

DISCUSSION

The presence of the endophyte *A. coenophialum* in tall fescue negatively influences populations of *P. scribneri.* Field populations of *P. scribneri* were reduced from 224 nematodes per 100 ml in E− pastures to two nematodes per 100 ml of soil when pasture E+ plant incidence was 75% (27). In our study, numbers of *P. scribneri* did not increase substantially on E− plants during the incubation period. The nematode was practically eliminated on E+ plants.

Soil populations of *H. pseudorobustus,* unlike those of *H. dihystera* (20), did not appear to be differentially affected by E+ and E− plants. This difference may be due to innate differences in the physiology of the nematodes or to differences in experimental method. Our data were highly variable, and it is possible that this variability masked a treatment effect. Also, if the degree of semiendoparasitism of *H. pseudorobustus* were higher on E− than on E+ plants, then a higher proportion of the population would be localized in the roots. Our extraction techniques did not target possible root-inhabiting *H. pseudorobustus.*

Differences in the sensitivity of the three nematodes to the presence of the endophytic fungus may be the result of one or more inhibitory compounds. Such compounds could be produced by the fungus and then translocated to the roots or produced by the plant in response to a signal. The presence of a nematicidal compound in tall fescue roots has not been established, but production of toxic compounds in the interaction between *A. coenophialum* and tall fescue has been well documented (29). Ergot peptide alkaloids, believed to be the primary factors in fescue toxicosis (2,18,29) and possibly involved in insect resistance (4), have not been found in the roots of tall fescue plants grown under the conditions used in these experiments (K. D. Gwinn, *unpublished*). Saturated pyrrolizidines (i.e., loline and its derivatives), which have been shown to be insecticidal (14),

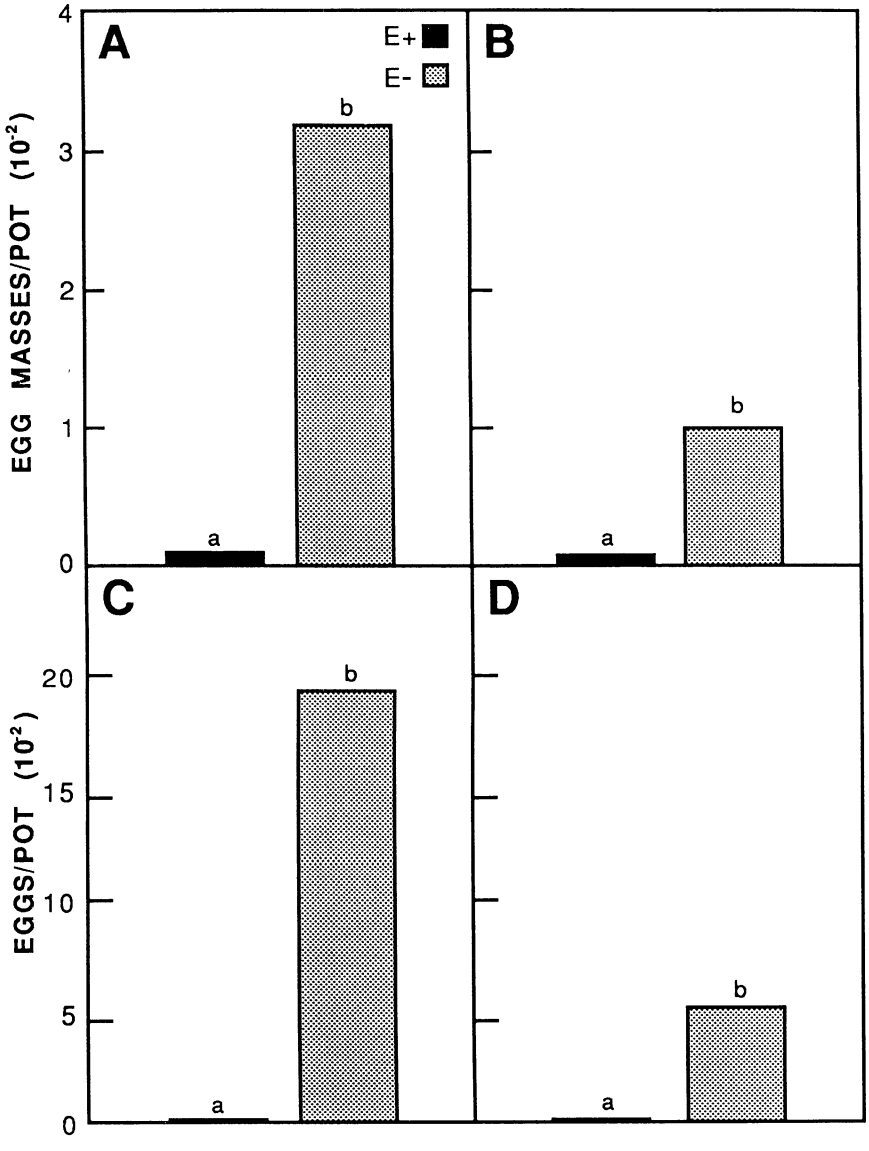

Fig. 3. Mean numbers of *Meloidogyne graminis* on endophyte-infected (E+) and endophyte-free (E−) tall fescue. **A and C,** experiment 1; **B and D,** experiment 2. Columns headed by different letters are different at $P = 0.05$ (using an independent *t* test).

occur in very low concentrations in the roots of E+ tall fescue (K. D. Gwinn, *unpublished*). Concentrations of other compounds such as perloline (29) in the roots are not known.

If inhibitory compounds are important in reducing the populations of some nematodes but not others, then nematodes may either vary in sensitivity to the compounds or receive different levels because of their feeding habits and life cycles. For example, *P. scribneri,* which spends most of its life cycle embedded within the cortex, would receive higher doses of inhibitory compounds localized in the cortex than *H. pseudorobustus,* which feeds primarily ectoparasitically on both cortical and epidermal cells. Females of *M. marylandi* feed on giant cell complexes within the vascular tissues, with their neck regions extending through the entire radius of the root; therefore, *M. marylandi* would be exposed to compounds regardless of localization.

Populations of the undescribed species of *Meloidogyne* showed no differences in reproduction on white clover when E− or E+ tall fescue was intermixed with the clover. This suggests that E+ tall fescue inhibits only nematodes that use it as a food source.

In preliminary time course studies, *M. marylandi* appeared to be less successful entering E+ fescue roots and developing to maturity therein than in E− roots (E. C. Bernard, *unpublished*). Although this information does not preclude the involvement of inhibitory compounds in the interaction, it does suggest that other mechanisms may be involved. One possibility is that infection with the endophyte induces micromorphological changes within the root that differentially exclude some nematodes. Reduction of cortical cell number or size, for example, would limit feeding sites for nematodes such as *P. scribneri.* Another possibility is that nematodes are differentially attracted to E− roots.

Different mechanisms may be important for determining the sensitivity of various nematodes to E+ tall fescue. It is possible that sensitivity may be predicted by the feeding sites and behavior of the nematode. However, more species need to be examined to resolve this issue.

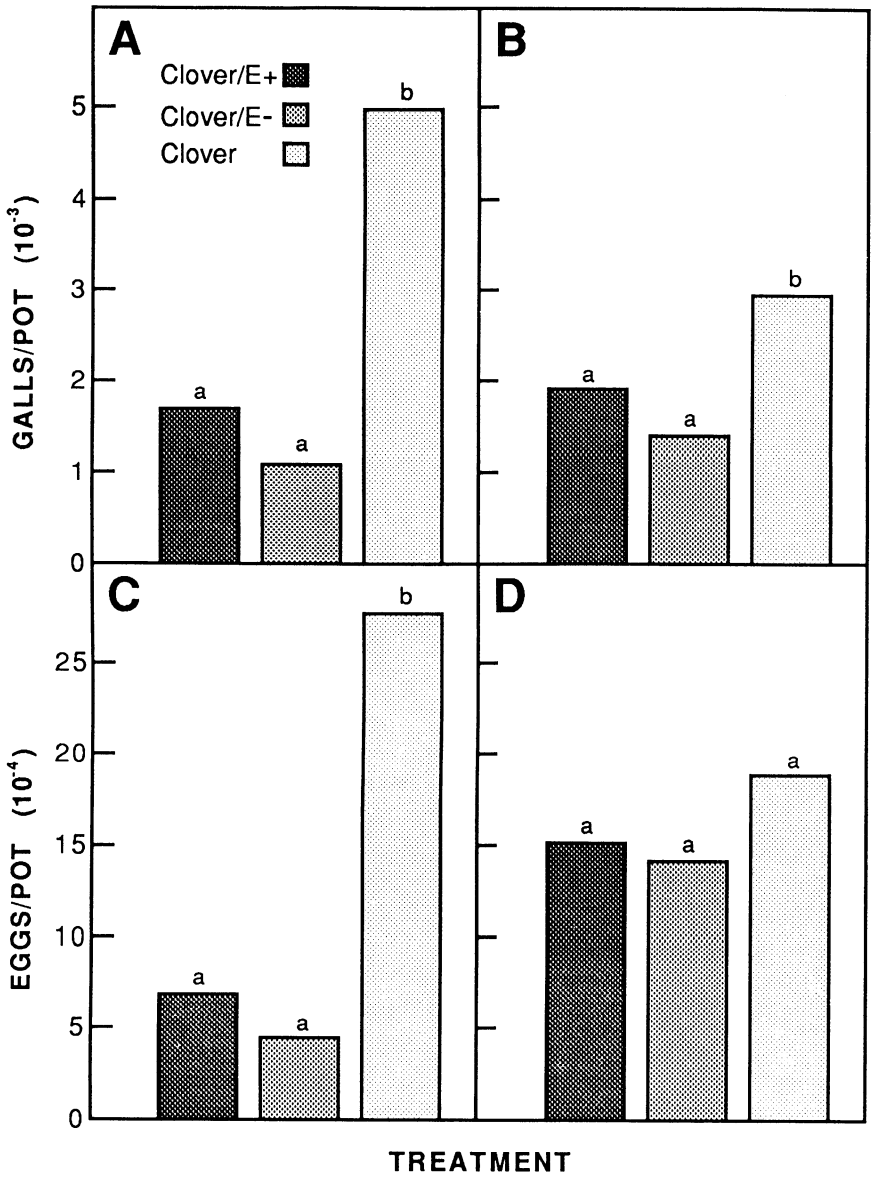

Fig. 4. Mean numbers of *Meloidogyne* sp. on white clover grown in the rhizosphere of endophyte-infected (E+) or endophyte-free (E−) tall fescue. **A and C,** experiment 1; **B and D,** experiment 2. Columns headed by the same letter do not differ at $P = 0.05$ (according to Duncan's new multiple range test for separation of means).

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LITERATURE CITED

1. Arachevaleta, M., Bacon, C. W., and Hoveland, C. S. 1989. Effect of the tall fescue endophyte on plant response to environmental stress. Agron. J. 81:83-90.
2. Bacon, C. W., Lyons, P. C., Porter, J. K., and Robbins, J. D. 1986. Ergot toxicity from endophyte-infected grasses: A review. Agron. J. 78:106-116.
3. Bernard, E. C. 1989. Host-parasite relationships of a new root-knot nematode on legumes. J. Nematol. 21:551.
4. Clay, K., and Cheplick, G. P. 1989. Effect of ergot alkaloids from fungal endophyte-infected grass on fall armyworm (*Spodoptera frugiperda*). J. Chem. Ecol. 15:169-182.
5. Conger, B. V., and McDaniel, J. K. 1983. Use of callus cultures to screen tall fescue samples for *Acremonium coenophialum.* Crop Sci. 23:172-174.
6. Fortuner, R. 1985. *Helicotylenchus pseudorobustus.* Descriptions of Plant-parasitic Nematodes, Set 8, No. 109. Commonw. Inst. Helminthol., St. Albans, Herts., England.
7. Gamborg, O. L., Murashige, T., Thorpe, T. A., and Vasil, I. K. 1976. Plant tissue culture media. In Vitro 12:473-478.
8. Golden, A. M. 1989. Further details and SEM observations on *Meloidogyne marylandi* (Nematode: Meloidogynidae). J. Nematol. 21:453-461.
9. Hardy, T. N., Clay, K., and Hammond, A. M., Jr. 1986. Leaf age and related factors affecting endophyte-mediated resistance to fall armyworm (Lepidoptera: Nocutidae) in tall fescue. Environ. Entomol. 15:1083-1089.
10. Hinton, D. M., and Bacon, C. W. 1985. The distribution and ultrastructure of the endophyte of toxic tall fescue. Can. J. Bot. 63:36-42.
11. Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Rep. 57:1025-1028.
12. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
13. Jepson, S. B. 1987. Identification of Root-Knot Nematodes (*Meloidogyne* Species). C.A.B. International, Wallingford, U.K. 265 pp.
14. Johnson, M. C., Dahlman, D. L., Siegel, M. R., Bush, L. P., Latch, G. C. M., Potter, D. A., and Varney, D. R. 1985. Insect feeding deterrents in endophyte-infected tall fescue. Appl. Environ. Microbiol. 49:568-571.
15. Kimmons, C. A., Gwinn, K. D., and Bernard, E. C. 1989. Reproduction of selected nematode species on endophyte-infected tall fescue. (Abstr.) Phytopathology 79:374.
16. Long, E. A., and Hilty, J. W. 1985. Occurrence of *Acremonium coenophialum* in tall fescue in Tennessee. Plant Dis. 69:467-468.
17. Loof, P. A. A. 1985. *Pratylenchus scribneri.* Descriptions of plant-parasitic nematodes. Set 8, No. 110. Commonw. Inst. Helminthol., St. Albans, Herts., England.
18. Lyons, P. S., Plattner, R. D., and Bacon, C. W. 1986. Occurrence of peptide and clavine ergot

alkaloids in tall fescue grass. Science 232:487-489.
19. Minton, N. A. 1965. Reaction of white clover and five other crops to *Pratylenchus scribneri.* Plant Dis. Rep. 49:856-885.
20. Pedersen, J. F., Rodriguez-Kabana, R., and Shelby, R. A. 1988. Ryegrass cultivars and endophyte in tall fescue affect nematodes in grass and succeeding soybean. Agron. J. 80:811-814.
21. Prestidge, R. A., Lauren, D. R., Van der Zijpp, S. G., and di Menna, M. E. 1985. Isolation of feeding deterrents in cultures of endophytes of perennial ryegrass and tall fescue. N. Z. J. Agric. Res. 28:87-92.
22. Read, J. C., and Camp, B. J. 1986. The effect of the fungal endophyte *Acremonium coenophialum* in tall fescue on animal performance, toxicity, and stand maintenance. Agron. J. 78:848-850.
23. Rebois, R. V., and Huettel, R. N. 1986. Population dynamics, root penetration, and feeding behavior of *Pratylenchus agilis* in monoxenic root cultures of corn, tomato, and soybean. J. Nematol. 18:392-397.
24. Reddick, B. B., and Collins, M. H. 1988. An improved method for detection of *Acremonium coenophialum* in tall fescue plants. Phytopathology 78:418-420.
25. Schenk, R. U., and Hildebrandt, A. C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 50:199-204.
26. Southey, J. F., ed. 1970. Laboratory Methods for Work with Plant and Soil Nematodes. Tech. Bull. 2. Minist. Agric. Fish. Food (G.B.). 148 pp.
27. Stuedemann, J. A., and Hoveland, C. S. 1988. Fescue endophyte: History and impact on animal agriculture. J. Prod. Agric. 1:39-44.
28. West, C. P., Izekor, E., Oosterhuis, D. M., and Robbins, R. T. 1988. The effect of *Acremonium coenophialum* on the growth and nematode infestation of tall fescue. Plant Soil 112:3-6.
29. Yates, S. G. 1983. Tall fescue toxins. Pages 249-273 in: CRC Handbook of Naturally Occurring Food Toxicants. M. Rechcigl, ed. CRC Press, Boca Raton, FL.
30. Zuckerman, B. M., Mai, W. F., and Harrison, M. B., eds. 1985. Plant Nematology Laboratory Manual. Univ. Mass. Agric. Exp. Stn., Amherst, MA. 212 pp.