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K.D. Gwinn, Major Professor

We have read this thesis and recommend its acceptance:

Ernest C. Bernard, Mark Windham, Don Dougall

Accepted for the Council:
Carolyn R. Hodges

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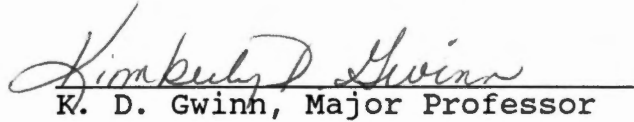

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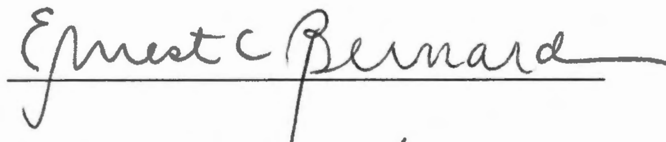

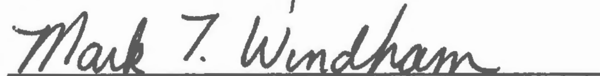

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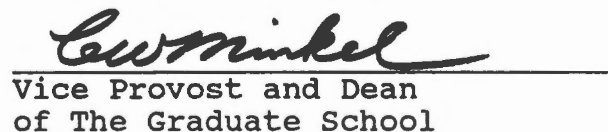

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Date May 26, 1989

NEMATODE RESISTANCE IN TALL FESCUE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Carol A. Kimmons

August 1989

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Thesis
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DEDICATION

This thesis is dedicated to my husband, sons, and mother, and to Dr. Kimberly Gwinn, my major professor.

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ABSTRACT

Tall fescue, Festuca arundinacea, an important forage grass in the southeastern United States, is frequently infected with Acremonium coenophialum, an endophytic fungus associated with fescue toxicosis in livestock. Endophyte-free (E-) tall fescue is more difficult to establish and are not as resistant to environmental stress and pests as endophyte-infected (E+) tall fescue. Greenhouse trials were conducted on three species of plant-parasitic nematodes with different feeding patterns to compare populations on E+ and E- tall fescue. Presence or absence of the endophyte was determined with PAS-ELISA. After 15 weeks, numbers of Pratylenchus scribneri, a migratory endoparasite, were significantly higher on E- than on E+ tall fescue roots. After 8 weeks, numbers of egg masses and eggs of Meloidogyne graminis, a sedentary endoparasite, were significantly higher on E- than on E+ tall fescue. Numbers of Helicotylenchus pseudorobustus, an ectoparasite, were not significantly different on E- and E+ tall fescue after 8 weeks. Numbers of an undescribed species of Meloidogyne, which is parasitic on legumes, were unaffected by presence of E+ or E- tall fescue in the rhizosphere. Results of these experiments suggest that substances within E+ tall fescue roots may be

inhibitory or toxic to some plant parasitic nematodes. These substances have not been identified. A bioassay using P. scribneri as the test organism was developed to determine toxicity of alkaloids and other compounds isolated from E+ tall fescue. Nematodes were exposed to test compounds for 48 hours; survivors were allowed to exit the test system for 72 hours and were counted. Ergotamine tartrate was more toxic to nematodes than saturated pyrrolizidines.

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I. INTRODUCTION

Tall fescue, Festuca arundinacea Schreb., is one of the most important forage crops in the southeastern United States, and is grown on over 1.4 million ha in Tennessee. Over 80 percent (Long and Hilty 1985) is infected with Acremonium coenophialum Morgan-Jones and Gams, an endophytic fungus which grows intercellularly within the leaf sheaths. Presence of the endophyte in tall fescue pastures is correlated with a number of symptoms in livestock referred to collectively as fescue toxicosis (Stuedemann and Hoveland 1988).

Fescue toxicosis results in production losses of millions of dollars each year. However, endophyte-infected (E+) tall fescue continues to be used for pasture and forage because of its tolerance of poor soils and severe climatic conditions. Endophyte-free (E-) tall fescue seed is available, but these pastures are more sensitive to environmental stress (West et al. 1988) and heavy grazing (Stuedemann and Hoveland 1988), possibly due to higher pest levels than in E+ tall fescue. Insect resistance in E+ tall fescue has been well documented (Johnson et al. 1985). West et al. (1988) found substantially lower populations of two species of plant-parasitic nematodes in E+ than in E- tall fescue field

plots. In greenhouse tests, depressed numbers of plant-parasitic nematodes were associated with E+ compared to E- tall fescue (Pedersen et al. 1988; Kimmons et al. 1989). Substances responsible for increased pest resistance in the rhizosphere of E+ tall fescue have not been identified, although alkaloids and other compounds isolated from roots, seeds, and stems of E+ tall fescue are known to have activity against arthropods and mammals.

The objectives of this study were 1) to compare populations of plant-parasitic nematodes on E- and on E+ tall fescue in greenhouse studies; 2) to determine whether the presence of roots of E+ tall fescue in the rhizosphere would affect populations of a species of nematode not parasitic on tall fescue; and 3) to develop a bioassay for determining toxicity of alkaloids and other substances isolated from E+ tall fescue.

II. LITERATURE REVIEW

TALL FESCUE

Festuca arundinacea Schreb., a cool season perennial bunch grass, was introduced into North America from Europe and was well established in the United States by 1871 (Stuedemann and Hoveland 1988). The 'Kentucky 31' cultivar was obtained from a farm in Menifee County, Kentucky, in 1931, by E. N. Fergus of the University of Kentucky. After its release in 1942, 'Kentucky 31' was rapidly and widely accepted as a result of its ease of establishment and maintenance in a wide range of soil types and climate (Stuedemann and Hoveland 1988). The United States Department of Agriculture and the Soil Conservation Service promoted the cultivar for stabilizing eroded soil, which further expanded its use. Stands tolerate poor drainage and drought conditions and persist almost indefinitely, even under poor grazing management (Stuedemann and Hoveland 1988). Approximately 14 million ha are now grown in the United States (Siegel et al. 1984).

FESCUE TOXICOSIS

Despite agronomic advantages and analysis as a high quality forage (Bush et al. 1979), tall fescue has become

associated with livestock performance problems. Cattle grazing on tall fescue pastures may experience a complex of problems referred to as fescue toxicosis (Robbins et al. 1972). Symptoms include poor weight gains, increased body temperature and breathing rate, nervousness, reduced conception rates, reduced lactation, and lameness (Bush et al. 1979; Read and Camp 1986).

TOXIC SUBSTANCES IN TALL FESCUE

Alkaloids, mycotoxins, and secondary metabolites from systemic fungi have been suspected as the cause of fescue toxicosis (Yates 1983). Toxic tall fescue forage produces several types of alkaloids, including ergopeptine alkaloids,a phenethylamine, diazaphenanthrines, a pyrrolopyrizineone, and seven types of lolines--norloline, loline, N-methyl loline, N-formyl norloline, N-acetyl norloline, N-formyl loline, and N-acetyl loline (Yates et al. 1988; Yates et al. 1985; Yates 1983). Yates et al. (1985) first detected ergopeptine alkaloids in tall fescue forage and noted that the symptoms of fescue toxicosis resemble those of ergotism in cattle. The ergot alkaloids are now considered a potential cause of fescue toxicosis (Bacon et al. 1986). Research is currently underway to determine if there is a causal relationship between

ergovaline, the most abundant alkaloid in fescue (Bacon et al. 1986), and fescue toxicosis.

THE ENDOPHYTE OF TALL FESCUE

An endophyte of tall fescue was discovered in 1941 in New Zealand (Stuedemann and Hoveland 1988). In 1977, Bacon isolated an endophyte from toxic tall fescue in the United States and suggested a relationship with fescue toxicosis (Bacon et al. 1977).

Morgan-Jones and Gams (1982) described the endophyte isolated from tall fescue as *Acremonium coenophialum* Morgan-Jones and Gams. The anamorphic state of *Epichloe typhina* Tulasne, *Sphacelia typhina* Sacc., was found to be similar to the fescue endophyte, but not identical to it.

The relationship between the endophytic fungus and fescue may be a form of mutualism or commensalism (Siegel et al. 1984). There is no known effect of the fungus on growth and reproduction of the grass. The fungus invades the developing seed and spreads into the endosperm. As the seedling germinates, hyphae grow between the cells, spreading throughout the plant tissue. There is no evidence that the endophyte penetrates into the root (Hinton and Bacon 1985). The leaf sheath contains the highest levels of fungus (Siegel et al. 1984). When flowering occurs, hyphae grow into the flower stem and

infect the seeds, which may contain almost as much fungal tissue as the leaf sheaths.

Dissemination of the endophyte can occur only as a result of seed production by the host grass. Use of infested seed for establishment of pastures is the probable cause of wide distribution of the endophyte in 'Kentucky 31' tall fescue (Long and Hilty 1985; Siegel et al. 1984). Estimates of incidence in the United States vary from 58 percent (Shelby and Dalrymple 1987) to 95 percent (Yates and Powell 1988).

RELATIONSHIP OF THE ENDOPHYTE TO PRESENCE OF TOXINS

Ergot alkaloids have been detected in all E+ tall fescue samples but in no E- samples (Yates et al. 1985; Lyons et al. 1986). Highest levels are found in sheaths, where the fungus mainly grows, but measurable levels are also found in uninfected blades, which suggests translocation of the alkaloids from point of origin (Lyons et al. 1986). Ergot alkaloids have not been found in tall fescue roots (Bacon et al. 1986).

There is a correlation between presence of the endophyte and saturated pyrrolizidine alkaloids (Jones et al. 1983); removal of the endophyte from tall fescue results in reduced levels of N-acetyl loline and N-formyl loline (Bush et al. 1982). Significantly greater levels

of N-acetyl loline and N-formyl loline were found in E+ tall fescue than in E- tall fescue from grazed paddocks (Belesky et al. 1987). Saturated pyrrolizidines have been found in all parts of the plant, including low levels in roots (Siegel, personal communication), which suggests translocation.

RESISTANCE OF ENDOPHYTE-INFECTED GRASSES TO GRAZERS

Production of alkaloids in E+ tall fescue may be an adaptive mechanism which inhibits feeding by grazers, both large (livestock) and small (arthropods) (Jones et al. 1983; Siegel et al. 1987b; Bacon et al. 1977; Read and Camp 1986; Johnson et al. 1985; Latch et al. 1985). Presence of the endophyte in tall fescue pastures is associated with reduced intake of forage and fescue toxicosis (Stuedemann and Hoveland 1988). Fescue toxicosis was first associated with endophyte-infected tall fescue pastures in the United States in 1973 (Stuedemann and Hoveland 1988). In 1981, Gallagher et al. isolated neurotoxins from perennial ryegrass, *Lolium perenne* L., that elicited mammalian responses corresponding to a disease of livestock referred to as ryegrass staggers. In 1982, Gallagher et al. found an association between ryegrass staggers and high levels of an endophyte in perennial ryegrass.

Insect resistance has been reported in both endophyte-infected ryegrass and tall fescue (Johnson et al. 1985). Presence of an endophyte in *L. perenne* is associated with resistance to the Argentine stem weevil, *Listronotus bonariensis* Kuschel. Mechanism of this resistance involves chemicals produced either by the fungus or by the ryegrass in response to invasion by the fungus (Stewart 1985). The aphid, *Rhopalosiphum padi* L., avoids E+ tall fescue plants, and settles on E- plants. The aphid is also deterred from seedlings grown from E+ tall fescue seed, although the presence of the fungus cannot yet be detected. This suggests that a deterrent factor is produced early in the growth of the plant and is translocated (Latch et al. 1985). In feeding preference tests on tall fescue using *R. padi* and *Schizaphis graminum* Rondani, the greatest deterrent activity was located in seed extracts containing the highest levels of saturated pyrrolizidines (Johnson et al. 1985).

Substances which result in insect deterrence in ryegrass differ from the substances responsible for outbreaks of ryegrass staggers in livestock (Rowan and Gaynor 1986). In tall fescue, pest-deterrent activity may be associated with saturated pyrrolizidine alkaloids (Johnson et al. 1985) and livestock toxicity is tentatively associated with ergot alkaloids

(Bacon et al. 1986). This suggests the possibility of producing modified biotypes of grass endophytes which would provide pest resistance to infected grass but would not harm livestock (Rowan and Gaynor 1986; Siegel et al. 1987a).

RESISTANCE OF E+ TALL FESCUE TO DROUGHT AND NEMATODES

Presence of plant-parasitic nematodes in turf and pastures is associated with grass damage, lower production (Feldmesser et al. 1975), and reduced tall fescue forage yields (Minton 1965; Hoveland et al. 1975). Nematode injury to grasses is most significant under conditions of moisture stress and high temperatures (Eriksson 1972). West et al. (1988) conducted field trials to determine effects of water stress and endophyte-infection on herbage yields and nematode populations in 'Kentucky 31' tall fescue stands. E+ (75 percent infected) plots produced higher herbage yields than E- (0 percent infected) plots in drought-stressed treatments. Leaf area per tiller was lower in E-plots at all irrigation levels; enhanced leaf senescence occurred under drought stress. Populations of two plant-parasitic nematodes were higher in the E- plots suggesting an association between nematode resistance and enhanced drought tolerance in E+ tall fescue. Higher populations of plant-parasitic nematodes have been found

in association with E- than with E+ tall fescue in greenhouse tests (Pedersen et al. 1988; Kimmons et al. 1989).

PLANT-PARASITIC NEMATODES

Tall fescue is a host for a number of plant-parasitic nematodes which may cause decreased growth and forage yields (Hoveland et al. 1975; Eriksson 1972). Pratylenchus scribneri Steiner is an obligate migratory endoparasite that can move freely within roots and in the rhizosphere (Loof 1985). This nematode has a wide host range and has been shown to reduce the forage yield of tall fescue (Minton 1965). In greenhouse tests, significantly lower populations of P. scribneri were found on E+ than on E- tall fescue roots after 15 weeks (Kimmons et al. 1989). Numbers were also lower in E+ field plots than in E- field plots (West et al. 1988). Helicotylenchus pseudorobustus (Steiner) Golden is an ectoparasite that lives in the rhizosphere and feeds with its body partially embedded in the root tissue. Although considered a weak pathogen, it may induce root necrosis, and interaction with other nematodes or pathogens may contribute to reductions in yields (Fortuner 1985).

Meloidogyne graminis (Sledge and Golden) Whitehead is a root-knot nematode that produces insignificant galling.

Adult females are attached to roots by the neck region and the body is exposed to the rhizosphere. Egg masses are located on the exterior of roots. Its host range is confined to members of the Family Poaceae and includes tall fescue (Jepson 1987). In greenhouse tests, significantly higher numbers of egg masses and eggs were found on E- than on E+ tall fescue roots (Kimmons et al. 1989). Meloidogyne sp. is an undescribed root-knot nematode. The sedentary females and the egg masses are located within the root and cause extensive galling. This endoparasitic nematode has a wide host range among members of the Family Fabaceae and does not reproduce on tall fescue (Bernard, unpublished). In greenhouse tests, numbers of this nematode on white clover, Trifolium repens L., were not affected by presence of E+ or E- tall fescue roots in the rhizosphere (Kimmons et al. 1989).

Populations of Tylenchorhynchus acutus Allen were lower in E+ field plots than in E- plots (West et al. 1988). In greenhouse tests, significantly fewer numbers of Paratrichodorus minor (Colbran) Siddiqi were associated with E+ than with E- tall fescue (Pedersen et al. 1988).

BIOASSAY TECHNIQUES

A large milkweed bug (Oncopeltus fasciatus Dallas) assay has been used to estimate ED_{50} values for alkaloids

found in fescue (Yates et al. 1988). Rhopalosiphum padi and S. graminum have been used in bioassays to confirm the presence of loline and peramine alkaloids in endophyte-infected grasses (Siegel et al. 1987a).

Moje (1959) developed a technique to estimate the toxic effects of halides on the motility of the citrus nematode Tylenchulus semipenetrans Cobb. A similar technique was used to determine the effect of glyceollin on the root-knot nematode Meloidogyne incognita (Kofoid and White) Chitwood (Kaplan et al. 1980a,b).

III. EFFECTS OF ENDOPHYTE-INFECTED TALL FESCUE ON POPULATIONS OF FOUR NEMATODE SPECIES

ABSTRACT

Greenhouse trials were conducted to compare the effects of endophyte-infected (E+) and endophyte-free (E-) tall fescue on numbers of four species of plant-parasitic nematodes with different feeding habits. Presence or absence of the endophyte, *Acremonium coenophialum*, was determined with PAS-ELISA. Numbers of *Pratylenchus scribneri*, a migratory endoparasite, were significantly higher on E- tall fescue roots than on E+ tall fescue roots. Numbers of *Helicotylenchus pseudorobustus*, an ectoparasite, were not significantly different on E+ and E- tall fescue roots. After eight weeks, numbers of egg masses and eggs of *Meloidogyne graminis*, a sedentary endoparasite, were significantly higher on E- than on E+ tall fescue roots. Numbers of an undescribed *Meloidogyne* species parasitic on legumes and found in a mixed white clover-tall fescue pasture, were unaffected by the presence of E+ or E- tall fescue roots in the rhizosphere.

INTRODUCTION

Tall fescue, *Festuca arundinacea* Schreb., is one of the most important forage crops in the southeastern United

States, and is grown on over 1.4 million ha in Tennessee (Long and Hilty 1985). Tall fescue is drought-resistant and tolerates a wide range of soil and climate conditions. Over 80 percent of the tall fescue in Tennessee is infected with Acremonium coenophialum Morgan-Jones and Gams, an endophytic fungus that grows intercellularly in the leaf sheath (Long and Hilty 1985). The relationship between the host grass and the fungus is believed to be symbiotic. 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 referred to collectively as fescue toxicosis (Stuedemann and Hoveland 1988; Bacon et al. 1977). Fescue toxicosis results in yearly economic losses of millions of dollars (Stuedemann and Hoveland 1988) 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 has lower resistance to heavy grazing (Stuedemann and Hoveland 1988) and drought (Read and Camp 1986; West et al. 1988).

Recent research suggests that increased stress tolerance in E+ tall fescue may be due in part to greater pest resistance. Presence of A. coenophialum in tall fescue is related to deterrence of feeding by the aphids,

Rhopalosiphum padi L. and Schizaphis graminum Rondani (Johnson et al. 1985). Populations of two plant-parasitic nematodes, Pratylenchus scribneri Steiner and Tylenchorhynchus acutus Allen, are reduced in E+ tall fescue field plots as compared to E- plots (West et al. 1988). In greenhouse tests, significantly lower numbers of Paratrichodorus minor (Colbran) Siddiqi were associated with E+ than with E- tall fescue (Pedersen et al. 1988).

No previous studies have compared the effects of E+ tall fescue on nematodes with different feeding patterns. The objectives of this study were 1) to compare numbers of P. scribneri, Helicotylenchus pseudorobustus (Steiner) Golden, and Meloidogyne graminis (Sledge and Golden) Whitehead on E+ and E- tall fescue roots after a period of growth in the greenhouse and 2) to determine whether the presence of E+ tall fescue roots in the rhizosphere would affect numbers of an undescribed species of Meloidogyne which is parasitic on white clover, Trifolium repens L.

Pratylenchus 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 reduce the forage yield of tall fescue (Loof 1985; Minton 1965). Helicotylenchus pseudorobustus is an ectoparasite that lives in the rhizosphere and feeds with its body partially embedded in the root tissue. Although

considered a weak pathogen, its presence may induce root necrosis, and interaction with other nematodes or pathogens may contribute to reductions in yields (Fortuner 1985). *Meloidogyne graminis* is a root-knot nematode which produces insignificant galling. Adult females are attached to the roots by the neck region and the body is exposed to the rhizosphere. Egg masses are also located on the exterior of roots. Its host range is confined to members of the Family Poaceae and includes tall fescue (Jepson 1987).

Meloidogyne sp. is an undescribed root-knot nematode. Both the sedentary females and the egg masses are located within the root and cause extensive galling. This endoparasitic nematode has a wide host range among members of the Family Fabaceae but does not reproduce on tall fescue (Bernard, *unpublished*).

Preliminary findings from this study have been reported (Kimmons et al. 1989).

MATERIALS AND METHODS

Plants. 'Kentucky 31' tall fescue plants were grown from seeds obtained from one seed lot. Single seeds were planted in individual peat pots. After one month's growth in the greenhouse, one tiller from each plant was tested for presence of *A. coenophialum* with an indirect protein-A

sandwich enzyme-linked immunosorbent assay (Reddick and Collins 1988). Plants with three consistent evaluations (E+ or E-) were divided into sets of three to four tillers and planted in 500-cm^3 steam-sterilized sand-soil mix in 7-cm diameter clay pots.

For the experiments with Meloidogyne sp., which is parasitic on legumes, 10 E+ or E- tillers were planted around the periphery of 20-cm diameter plastic pots. White clover, T. repens, was planted in the center of the pots. Clover and fescue were allowed to grow together for one month before inoculum was added.

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

Nematodes. The inoculum of P. scribneri was obtained from R. N. Huettel (Plant Protection Institute, USDA-ARS NER, Beltsville, MD 20705) on 'I. O. Chief' corn (Zea mays L.) root explants and was increased on 'Forager' tall fescue callus tissue grown in SH-30 medium (Schenk and Hildebrandt 1972) with 30 μM dicamba (3,6-dichloro-0-anisic acid, Velsicol Chemical Corp.),

30 g/l sucrose, 9 g/l Difco bacto-agar, and 1 g/l inositol, and adjusted to pH 5.6 (modifications are as reported by Conger and McDaniel 1983, for growth of fescue callus tissue); or on 'Seneca Chief' corn (Zea mays L.) root explants grown in Gamborg's B-5 medium without auxins or cytokinins (Gibco Laboratories) with 10 g/l Difco bacto-agar and adjusted to pH 5.7 (Gamborg et al. 1976; Rebois and Huettel, 1986). Nematodes for inoculation were obtained by a modified Seinhorst mist apparatus (Southey 1970). Callus was placed on Nitex™ nylon screen, with 23 µm pores (Tetko, Inc., 420 Saw Mill River Road, Elmsford, NY), which was glued between two 1-cm long pieces of 10-cm diameter PVC (polyvinyl chloride) pipe. This apparatus was placed in a funnel (10-cm diameter). The stem of the funnel was suspended in a 250-ml flask. A fine mist was sprayed over the callus for 10 sec every 10 min for a 2 wk period. Nematodes emerged from the callus, washed through the screen, and were collected at 24-hr intervals from water in the flask. Nematodes were counted at 35x magnification. A 10-ml aqueous suspension of 1200 nematodes/pot in Experiment 1 and 2000/pot in Experiment 2 was pipetted into five 1-cm^3 holes spaced evenly around E- or E+ tall fescue plants and 3 cm away from the tillers. The treatments in each experiment were replicated six times.

After 15 weeks, plants were removed from the pots. Nematodes were extracted from the roots by a modification of the centrifugal-flotation technique (Jenkins 1964). Roots were washed, cut into 1-cm pieces, and macerated in a Waring Blendor[tm] with 100 ml water for three 10-sec intervals at high speed (approximately 12,600 rpm). The suspension was placed in four 50-ml centrifuge tubes and centrifuged for eight min at 400 x g. Supernatant was decanted onto a 200-mesh (75 μm pores) sieve to collect any suspended material. Pellets were suspended in a sucrose solution (454 g sucrose/l water) and centrifuged again for 5 min at 400 x g. Supernatant was decanted onto a 200-mesh sieve nested in a 500-mesh (25 μm pores) sieve. Nematodes were rinsed from the 500-mesh sieve into a counting tray and counted. Macerate remaining on the 200-mesh sieve was added to the pellet remaining in the tubes and to material saved from the first centrifugation. This was placed in the mist chamber as described above. Nematodes were collected and counted at 35x magnification at 24-hr intervals for seven days.

Nematodes were also extracted from a 100 cm^3 subsample of the soil in each pot by centrifugal-flotation (Jenkins 1964). The subsample of soil was placed in a 1000 ml plastic beaker. Water was added to bring total volume to 800 ml. Solution was stirred for 20 sec and allowed to

settle for 40 sec. Solution was decanted onto an 80-mesh (180 μm pores) sieve over a 400-mesh (38 μm pores) sieve. Sieves were rinsed. Debris and nematodes on the 400-mesh sieve were washed through a funnel into a centrifuge tube and centrifuged at 400 x g for 4 min. Supernatant was decanted. Sucrose solution (454 gm sucrose/l water) was added to pellet and stirred. Tube was centrifuged for 60 sec at 400 x g. Sugar solution/nematode suspension was decanted onto a 500-mesh (25 μm pores) sieve and rinsed with water. Nematodes were rinsed from this sieve into a 100-ml beaker, then placed in a counting dish and counted. Numbers obtained from the mist extraction and soil were added to numbers collected from sieves and recorded.

The inoculum of _H_. _pseudorobustus_ was collected from a fescue pasture at Ames Plantation in West Tennessee and maintained on sunflower, _Helianthus_ _annuus_ L., in the greenhouse. Nematodes for the experiments were extracted from the soil with centrifugal-flotation as described above and counted at 35x magnification. A 10-ml aqueous suspension containing 1000 nematodes was pipetted into five 1-cm^3 holes evenly placed around the E- or E+ tillers in each pot at a distance of 3 cm. Treatments in each experiment were replicated eight times. After 8 weeks in the first experiment and 10 weeks in the second experiment, nematodes were extracted from a 100 cm^3

subsample of the soil in each pot with centrifugal-flotation (as described above), a subsample was counted, and numbers were recorded.

The inoculum of M. graminis was collected from bermuda grass (Cynodon dactylon L.) turf in Knoxville, TN, and was maintained on bermuda grass in the greenhouse. Eggs for the experiments were obtained by placing roots in 1 percent sodium hypochlorite and shaking the solution intermittently for four minutes. The suspension was decanted onto a 200-mesh (75 μm pores) sieve nested in a 500-mesh (25 μm) sieve. Eggs were collected from the 500-mesh sieve (Hussey and Barker 1973) and a subsample was counted at 40x magnification. A 10-ml suspension of 3400 eggs in Experiment 1 and 5000 in Experiment 2 was poured onto 250 cm^3 sand-soil mixture in 7-cm diameter clay pots. E+ or E- tillers were placed in the pots and sand-soil mixture was added to fill the pots. There were eight replicates in Experiment 1 and five replicates in Experiment 2.

After two months, plants were removed from the soil. Egg masses were stained by soaking roots 15 minutes in a solution of phloxine-B (0.15 g/l tap water) (Sigma Chem. Co., St. Louis, MO) (Zuckerman et al. 1985), and counted at 10x magnification. Roots were then cut into 1-cm

pieces and eggs extracted by the process described above. A subsample of eggs was counted at 40x magnification.

An undescribed species of Meloidogyne was collected from a mixed E- tall fescue-clover pasture at Ames Plantation in West Tennessee. Meloidogyne sp. was maintained in the greenhouse on white clover. Eggs for inoculum were extracted by macerating the roots in a Waring Blendor™ for three 10-sec intervals at high speed (approximately 12,600 rpm). The suspension was poured through a 200-mesh (75 μm pores) sieve nested in a 500-mesh (25 μm) sieve. Eggs were collected from the 500-mesh sieve and a subsample counted at 40x magnification.

In Treatment 1, 10 E+ tillers were planted around the periphery of 20-cm diameter pots. White clover was planted in the center of the pots. In Treatment 2, 10 E- tillers were planted around the periphery and white clover planted in the center. In Treatment 3, white clover was planted in pots without tall fescue. After one month, a 10-ml aqueous suspension of 4000 eggs was pipetted into five 1-cm^3 holes placed randomly in the rhizosphere of the clover in each pot. Treatments were replicated eight times in the first experiment and five times in the second experiment.

After 10 (Experiment 1) to 14 (Experiment 2) weeks, clover roots were harvested, washed, and weighed. Galls

were counted on a subsample of the roots at 10x magnification. The entire root system was then cut into 1-cm pieces, macerated, and eggs collected and counted as described above.

In order to determine if this undescribed species of Meloidogyne was able to survive on tall fescue, E+ and E- plants were inoculated with an aqueous suspension of 4000 eggs of Meloidogyne (obtained from white clover as described above). A 10-ml suspension was placed in five 1-cm^3 holes spaced evenly around the tillers of fescue in 7-cm diameter clay pots. After 8 wks, plants were removed from soil, washed, and examined at 10x magnification. No galls were observed.

Statistics. Data were tested for significance using an independent t-test for experiments with P. scribneri, H. pseudorobustus, and M. graminis and analysis of variance 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, distribution was not normal, and data were transformed by log (x + 1) before analysis of variance.

RESULTS

Pratylenchus scribneri. E- treatment (Fig. III-1): In Experiment 1, numbers of nematodes at the end of the

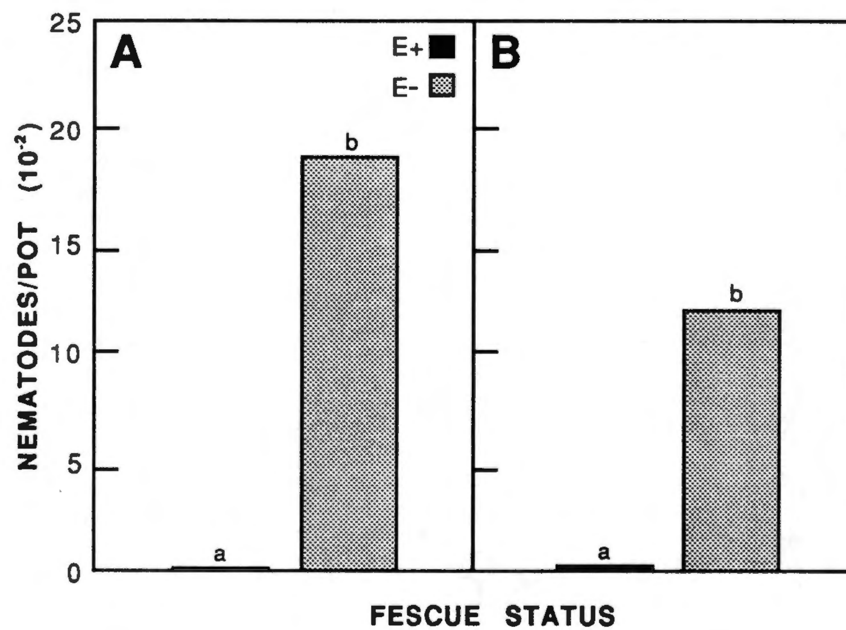

Fig. III-1. Mean numbers of Pratylenchus scribneri on endophyte-infected (E+) and endophyte-free (E-) tall fescue. **(A)**, Experiment 1; **(B)**, Experiment 2. Columns headed with different letters are significantly different at P = 0.05 (using an independent t-test).

experiment were higher than inoculum level of 1200 in four of six replicates, with a high of 5222 and a mean of 1890. In Experiment 2, final numbers of nematodes were higher than inoculum level of 2000 in one replicate. The highest number of nematodes was 2818 and the mean was 1182, which was less than the inoculum level.

E+ treatment (Fig. III-1): In Experiment 1, no nematodes were found on any of the six replicates. In Experiment 2, none were found on four replicates. The remaining replicates contained 10 and 40.

In both Experiments 1 and 2, final numbers of nematodes per replicate were significantly (P = 0.05) lower on E+ plants than on E- plants.

Helicotylenchus pseudorobustus. E- treatment (Fig. III-2): In Experiment 1, numbers of nematodes after 8 wks ranged from 144 to 1500 with a mean of 960. Numbers increased from inoculum level of 1000 on three of six replicates. In Experiment 2, final numbers after 10 wks ranged from 280 to 4400 with a mean of 1218. Numbers increased from inoculum level on four of six replicates.

E+ treatment (Fig. III-2): In Experiment 1, final numbers of nematodes ranged from 280 to 1140 with a mean of 735. Numbers increased from inoculum level in one replicate. In Experiment 2, final numbers ranged from 320

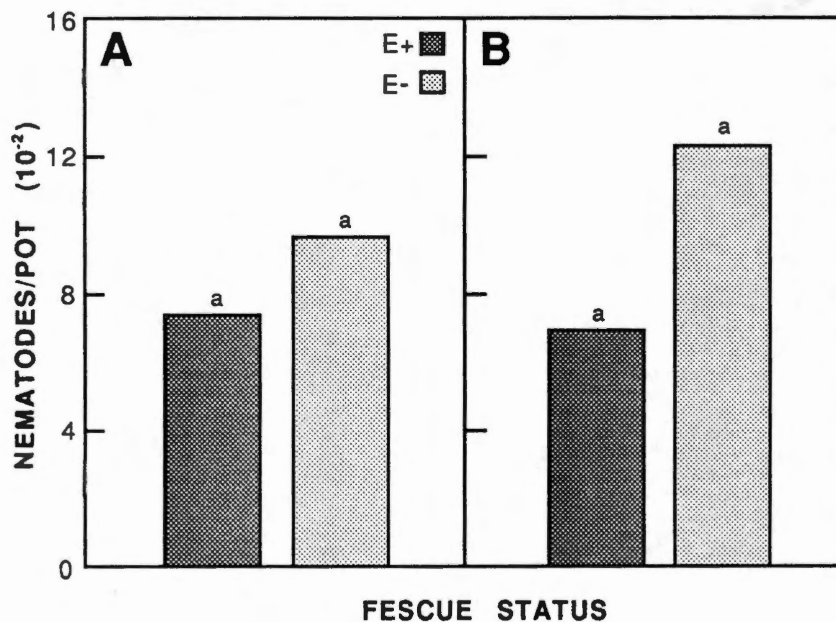

Fig. III-2. Mean numbers of <u>Helicotylenchus pseudorobustus</u> on endophyte-infected (E+) and endophyte-free (E-) tall fescue. **(A)**, Experiment 1; **(B)**, Experiment 2. Columns headed with the same letter are not significantly different at P = 0.05 (using an independent t-test).

to 1460 with a mean of 688. Numbers increased from inoculum level in two of six replicates.

Final numbers on E+ and E- treatments were not significantly (P = 0.05) different in Experiment 1 or 2.

Meloidogyne graminis. E- treatment (Fig. III-3): In Experiment 1, numbers of egg masses on roots ranged from 142 to 562 with a mean of 315. Numbers of eggs extracted from roots ranged from 3600 to 33,600 with a mean of 19,240. In Experiment 2, numbers of egg masses ranged from 11 to 271 with a mean of 99. Numbers of eggs ranged from 312 to 16,000 with a mean of 5122.

E+ treatment (Fig. III-3): In Experiment 1, numbers of egg masses ranged from 0 to 24. There were no egg masses in two of the eight replicates. Numbers of eggs ranged from 0 to 200. In six of eight blocks, there were no eggs. The mean was 43. In Experiment 2, mean number of egg masses was 5. Mean number of eggs was 16.

In both Experiments 1 and 2, final numbers of egg masses and eggs were significantly lower in E+ treatments than in E- treatments.

Meloidogyne sp. E- treatment (Fig. III-4): In Experiment 1, there was a mean of 1099 galls on clover grown with E- tall fescue. Mean number of eggs extracted was 45,400. In Experiment 2, mean number of galls counted was 1402. Mean number of eggs extracted was 140,800.

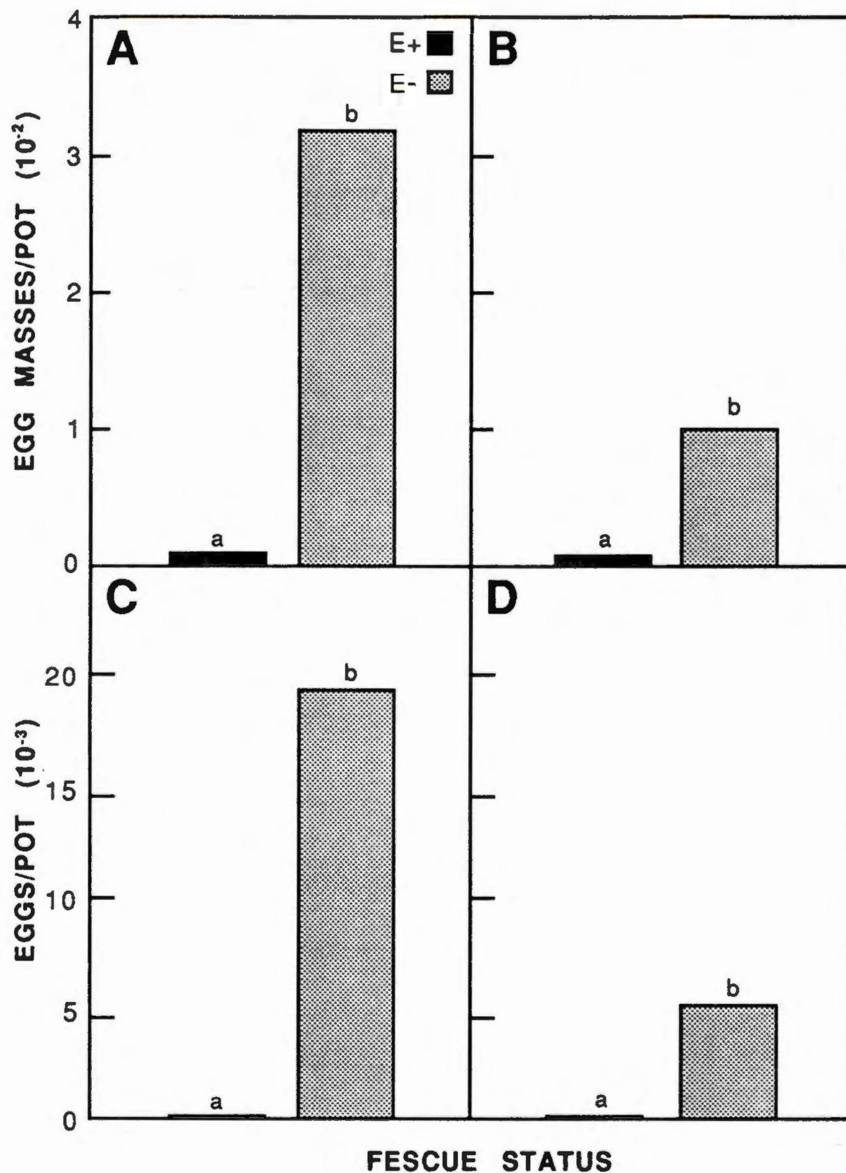

Fig. III-3. Mean numbers of Meloidogyne graminis on endophyte-infected (E+) and endophyte-free (E-) tall fescue. **(A)** and **(C)**, Experiment 1; **(B)** and **(D)**, Experiment 2. Columns headed with different letters are not significantly different at P = 0.05 (using an independent t-test).

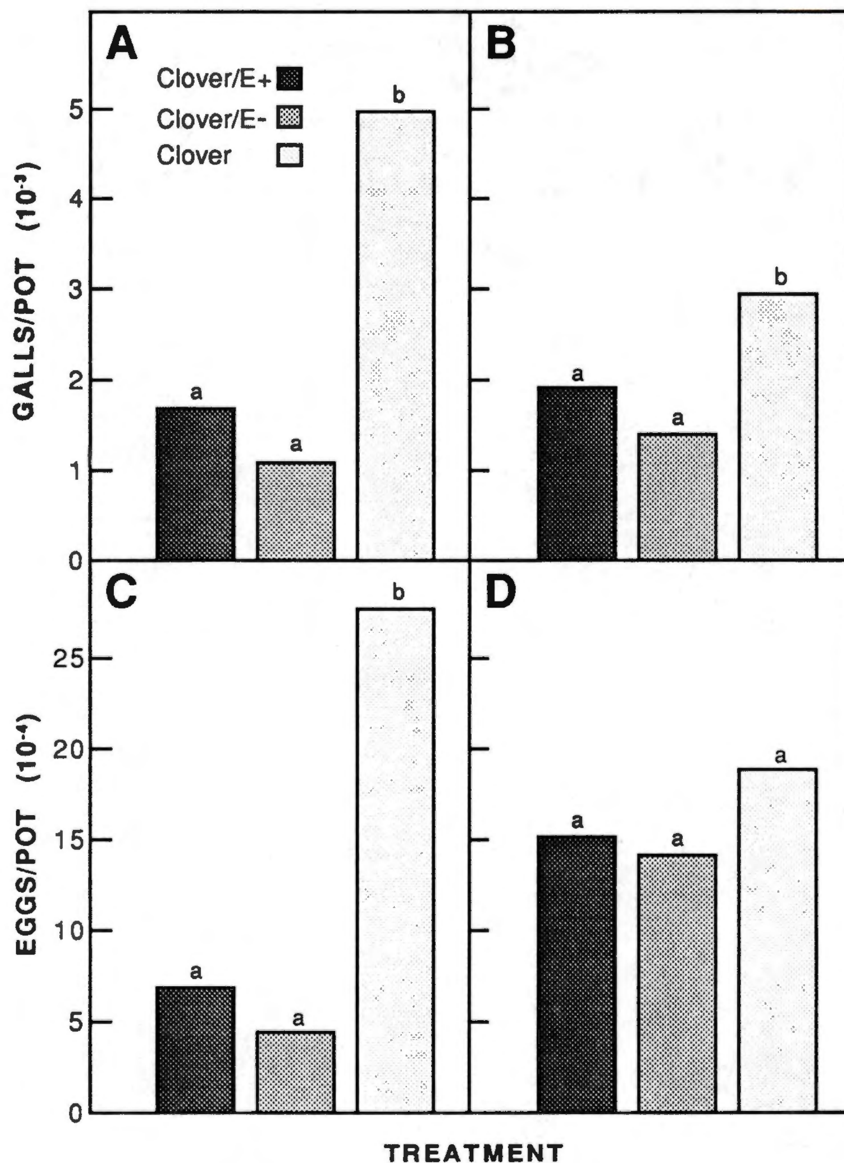

Fig. III-4. Mean numbers of _Meloidogyne_ sp. on white clover grown in the rhizosphere of endophyte-infected (E+) or endophyte-free (E-) tall fescue. **(A)** and **(C)**, Experiment 1; **(B)** and **(D)**, Experiment 2. Columns headed by the same letters are not significantly different at P = 0.05 (according to Duncan's New Multiple-Range Test for separation of means).

E+ treatment (Fig. III-4): In Experiment 1, a mean of 1679 galls were counted on clover roots grown with E+ fescue. Mean number of eggs extracted was 68,458. In Experiment 2, a mean of 1896 galls was counted on clover roots. Mean number of eggs extracted was 150,000.

Clover treatment (Fig. III-4): In Experiment 1, mean number of galls on clover grown alone was 4898. Mean number of eggs extracted was 272,588. In Experiment 2, mean number of galls was 2924. Mean number of eggs extracted was 186,600.

There was no significant difference in numbers of eggs or galls in E+ and E- treatments. Numbers on clover grown alone were significantly higher; this is probably due to lack of competition from fescue roots under greenhouse conditions.

DISCUSSION

These data suggest that the presence of *A. coenophialum* in tall fescue affects numbers of some plant-parasitic nematodes. However, the responses of tested nematodes to E+ tall fescue are related to their feeding patterns. Numbers of *P. scribneri*, which lives and feeds mainly in the cortex (Loof 1985), decreased to minimal levels in E+ tall fescue. Factors responsible for this lack of host suitability of E+ tall fescue are not known.

Insect deterrence in E+ tall fescue (Johnson et al. 1985) and the presence of alkaloids in the roots (Siegel, personal communication) suggest that the cortex cells of the roots contain substances which are either toxic to P. scribneri or inhibit development. Morphological changes in E+ tall fescue roots affecting the cortex cells could result in decreased feeding sites. Histological studies are currently being conducted to investigate this factor.

The adult females of M. graminis attach permanently to vascular cells before producing egg masses. They are directly exposed to substances which are translocated by the vascular system. These substances may result in poor development and/or reproduction of the nematodes.

Helicotylenchus pseudorobustus, an ectoparasite, feeds on the endodermis, pericycle, and cortex cells (Fortuner 1985). Endodermis and pericycle would provide feeding sites if there were a decrease in numbers of cortex cells in E+ plants due to presence of the endophyte. Helicotylenchus pseudorobustus does not remain within the roots and would not be as affected by toxic substances in the roots.

If toxic substances in the roots diffused into the rhizosphere, there would possibly be a significant effect on populations of Meloidogyne sp. and H. pseudorobustus. Lack of an effect on these nematodes suggests 1) toxic or

inhibitory substances in the root do not move into the rhizosphere; 2) substances move into the rhizosphere but lose their biological activity through leaching, bacterial action, or biochemical reactions with soil compounds; or 3) toxic substances diffuse into the rhizosphere but *H. pseudorobustus* and *Meloidogyne* sp. are not affected by the substances.

Further studies on 1) sensitivity of plant-parasitic nematodes to E+ tall fescue root compounds and root diffusates and 2) morphological changes in E+ roots are necessary to determine causes of decrease in numbers of some nematodes on E+ plants as compared to E- plants.

If nematode resistance is due to presence of toxic compounds in E+ tall fescue roots, these compounds may not be the same chemicals responsible for fescue toxicosis. Both ergot alkaloids and saturated pyrrolizidines are found in E+ tall fescue (Belesky et al. 1987; Siegel et al. 1987b). The ergot alkaloids, now considered a probable cause of fescue toxicosis (Yates 1983; Bush et al. 1979), have not been found in tall fescue roots (Bacon et al. 1986). Saturated pyrrolizidines are present in E+ tall fescue roots (Siegel, *personal communication*), have been shown to have low mammalian toxicities (Bush et al. 1979; Yates 1983), and are implicated in insect deterrence (Johnson et al. 1985). This suggests the possibility of

developing an endophyte-infected tall fescue which continues to maintain pest resistance but does not produce chemicals deleterious to livestock.

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IV. BIOASSAY FOR ALKALOIDS OF ENDOPHYTE-INFECTED TALL FESCUE

ABSTRACT

Pratylenchus scribneri, a nematode whose populations are lower on tall fescue infected with the endophytic fungus, *Acremonium coenophialum*, than on endophyte-free tall fescue, was used to determine relative toxicities of alkaloids produced in the tall fescue-*A. coenophialum* interaction. Nematodes were exposed to test compounds for 48 hours; surviving motile nematodes were then allowed to exit the test system for 72 hours and were counted. Ergotamine tartrate was more toxic to *P. scribneri* than saturated pyrrolizidines. Loline dihydrochloride was the most toxic of tested saturated pyrrolizidines.

INTRODUCTION

Alkaloids have been isolated from tall fescue (*Festuca arundinacea* Schreb.) infected with the endophytic fungus, *Acremonium coenophialum* Morgan-Jones and Gams. These include lolines, perloline, perlolidine (Bush et al. 1979; Yates 1983), and ergopeptine alkaloids (Yates et al. 1985; Lyons et al. 1986). Saturated pyrrolizidines have low oral toxicities in mammals (Bush et al. 1979). The ergot alkaloids are vasoconstrictors and have severe

physiological effects (Cordell 1981).

At least six species of insects are reported to be affected by endophyte-infected (E+) tall fescue (Siegel et al. 1987b). Saturated pyrrolizidine alkaloids are implicated in insect feeding deterrence (Johnson et al. 1985; Latch et al. 1985). Latch et al. (1985) suggested use of the aphid, Rhopalosiphum padi L., for detection of the endophyte. Rhopalosiphum padi and Schizaphis graminum Rondani have been used in bioassays to confirm the presence of loline and peramine alkaloids in endophyte-infected Festuca and Lolium species (Siegel et al. 1987a). A large milkweed bug (Oncopeltus fasciatus Dallas) assay has been used to estimate ED_{50} values for fescue alkaloids (Yates et al. 1988).

A bioassay for fescue alkaloids using species of nematodes known to be affected by E+ tall fescue can provide information on relative toxicities and modes of action. Moje (1959) developed a technique to estimate the toxic effects of halides on the motility of Tylenchulus semipenetrans Cobb. A similar technique was used to determine the effect of glyceollin on Meloidogyne incognita (Kofoid and White) Chitwood (Kaplan et al. 1980a,b). The objective of this study was to develop a bioassay for determination of lethality of fescue alkaloids. The test organism used was

Pratylenchus scribneri Steiner, a plant-pathogenic nematode which has been shown to reduce the yield of tall fescue (Minton 1965) and whose numbers are significantly lower on E+ than on E- tall fescue (Kimmons et al. 1989).

MATERIALS AND METHODS

Apparatus. Vials. Glass, 21 mm x 50 mm, with snap-on caps (Kimble) (Fig. IV-1).

Filter cloth. Nitex[tm] nylon screen with 23 μm pores (Tetko Inc., Elmsford, NY).

Rubber rings. Sizes to attach nylon screen to vials and to suspend vials in counting trays.

Counting trays. Plastic, lidded, 75 mm x 33 mm, with counting grid (Fig. IV-2).

Test organism. Pratylenchus scribneri can be maintained in axenic cultures and has a long storage life at 0 C (Loof 1985). Original inoculum was obtained from R. N. Huettel (Plant Protection Institute, USDA-ARS NER, Beltsville, MD 20705) on 'Iochief' corn root explants (Zea mays L.). Populations were maintained on 'Seneca Chief' corn root explants (Zea mays L.) grown in Gamborg's B-5 medium without auxins or cytokinins (Gibco Laboratories), with 10 grams of Difco bacto-agar per liter, adjusted to pH 5.7 (Gamborg et al. 1976; Rebois and Huettel 1986) or on 'Forager' tall fescue callus tissue grown in SH-30

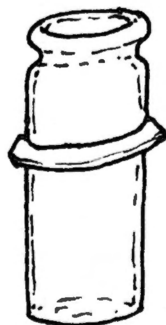

Fig. IV-1. Ten milliliter glass vial for exposure of nematodes to test solutions and controls. Adjustable rubber ring suspends inverted vial in counting tray.

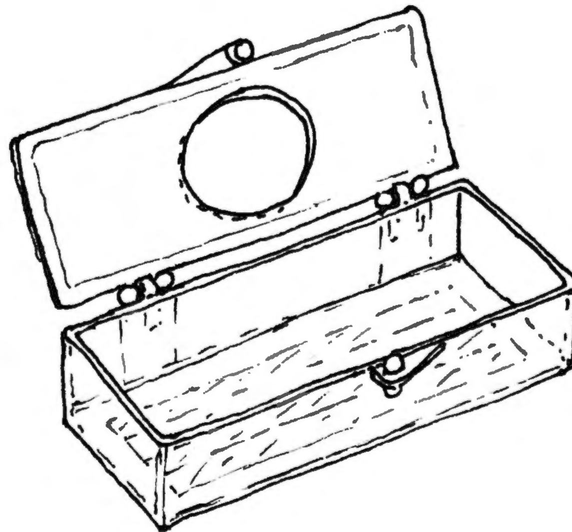

Fig. IV-2. Plastic counting tray with grid. Opening in lid was made with a heated pipe and allows vial to be suspended in tray.

medium (Schenk and Hildebrandt 1972) with 30 $\mu\underline{M}$ dicamba (3,6-dichloro-O-anisic acid, Velsicol Chemical Corp.), 1 g/l inositol, 30 g/l sucrose, and 9 g/l Difco bacto-agar, adjusted to pH 5.6 (modifications are as reported by Conger and McDaniel 1983, for growth of fescue callus tissue). Nematode suspensions for the bioassays were isolated from the cultures by the following procedure. Corn root explants or callus tissue infested with nematodes were macerated in a Waring Blendor^{tm} with 100 ml tap water for 20 seconds at high speed (approximately 12,600 rpm). The suspension was decanted onto a 400-mesh (38 μm pores) sieve in order to remove smaller juveniles. Nematodes were extracted from the macerate in a modified Baermann pan (Zuckermann et al. 1985). Nylon screen (Nitex^{tm}, 23 μm pores, from Tetko Inc., Elmsford, NY) was glued between two pieces of 10-cm diameter PVC (polyvinyl chloride) pipe. The screen was lined with two Kimwipes^{tm}. Macerate was placed on the Kimwipes^{tm}. This apparatus was set into a shallow pan. Water was added to the pan to a level just above the screen. Nematodes which moved through the screen were collected on a 400-mesh (38 μm) sieve and stored in tap water at 12 C for up to three days until use in bioassays.

Alkaloids. Loline dihydrochloride, N-formyl loline, N-acetyl loline, and N-methyl loline dihydrochloride were

obtained from Richard G. Powell, Research Leader, Bioactive Constituents Research, United States Department of Agriculture, Agricultural Research Service, Peoria, Illinois. Purity was determined by Richard J. Petroski using gas chromatography. Compounds were dissolved in methanol. Molar dilutions were made using methanol. N-acetyl loline and N-formyl loline were tested at concentrations ranging from 0.06 mM (N-acetyl loline) or 0.01 mM (N-formyl loline) to 5 mM. N-methyl loline dihydrochloride was tested at concentrations ranging from 8×10^{-5} mM to 5 mM. Loline dihydrochloride was tested at concentrations ranging from 0.1 mM to 4.0 mM. Controls contained the same percentages of methanol as the dilutions of test compounds (either 1 or 4 percent).

Millimolar quantities of ergotamine tartrate (Sigma Chem. Co., St. Louis, MO). were dissolved in a buffer (10 mM KH_2PO_4) with 1 percent (v/v) acetone, and adjusted to pH 5. Concentrations ranged from 0.010 mM to 0.333 mM. To determine effect of the tartrate ion, equal mM quantities of tartaric acid (Mallinckrodt, Paris, KY) were dissolved in the same solution. For the control, buffer with 1 percent acetone was used.

Procedure. Nematodes were suspended in tap water and placed in each vial. Sample size was 400-600 nematodes. Dilutions of compounds to be tested were added. The

maximum concentration of methanol used as the solvent in vials of test compounds was 4 percent. Controls for experiments with loline alkaloids contained the same percentages of methanol as the test vials. Controls for experiments with ergotamine tartrate contained the same buffers as the test vials. Final volume in each vial was 10 ml. Vials were capped with nylon screen, covered with aluminum foil, and placed on a shaker. In each experiment, there were either three or four replicates of each concentration.

Exposure time. Nematodes were placed in glass vials with the test compound for 48 hours. Vials were placed on a shaker in order to provide even aeration and distribution of all individuals. After 48 hours, vials were removed from the shaker and inverted in counting trays containing 8 ml water. Vials were positioned with the nylon screen below the surface of the water but above the bottom of the tray (Fig. IV-3). After 72 hours, vials were carefully removed from the trays. The number of nematodes in each tray was determined by counting a subsample at 35x magnification. The percentage of survival was determined by comparing the mean number at each concentration to the mean number in the control group. Some experiments were repeated.

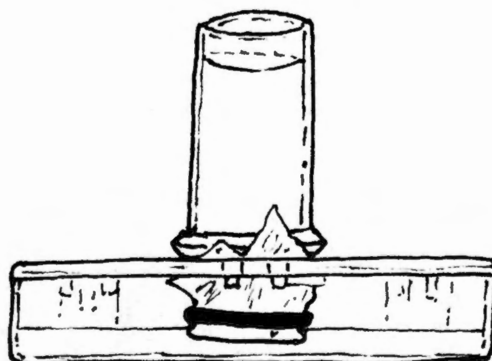

Fig. IV-3. Inverted vial is positioned with nylon screen below level of water and above base of tray.

RESULTS AND DISCUSSION

Determination of lethality. In this technique, only motile nematodes passed through the screen and were counted as survivors. In controls, over 80 percent of nematodes passed through the screen. Nematodes remaining in vials were either dead or immotile. Casual observation suggested that the majority of these were dead. They frequently exhibited abnormalities, such as vacuolated body cavities, exudates from stylets, lack of stylets, or body deformities.

A sample size of 400 to 600 was chosen after trials with 200, 400, 600, and 1000. In trials with 200, there was a high variation in results. Counting errors occurred when numbers exceeded 600.

Moje (1959) compared exposure times of 96, 120, and 240 hours and found little difference in mortality as a function of time. Kaplan et al. (1980a) used an exposure time of 24 hours in the vials; he then inverted vials into containers of the test solution and counted survivors after 24 hours. Vranken et al. (1985), in studies of the effects of heavy metals on free-living nematodes, found LC_{50} values to be extremely time dependent.

Inversion time. Inverting vials permits motile nematodes which have survived exposure to test compounds to move through the screens into the counting trays. In

preliminary experiments, test vials containing water suspensions of nematodes were inverted into counting trays. Numbers of nematodes in trays and numbers retained by the screen in test vials were counted after inversion for 24, 48, and 72 hours. After inversion for 24 hours, the maximum number of motile nematodes retained by the screen was 40. After 72 hours, the maximum number of retained motile nematodes in a vial was 10. To provide maximum passage of motile nematodes, a period of 72 hours was selected. With this technique, the number of nematodes which are alive and motile enough to move through the screen after the 48-hour exposure can be determined.

Effects of solvents. Acetone was used initially to determine the efficiency of the test system in separating motile and non-motile nematodes. A concentration of 10 percent acetone was lethal to over 50 percent of nematodes (Table IV-1). In methanol, mean survival rate at 10 percent concentration was 61 percent (Table IV-2).

Saturated pyrrolizidines. In N-acetyl loline (Table IV-3), there was no decrease in survival at concentrations up to 5 m<u>M</u>. In N-formyl loline (Table IV-4), mean

Table IV-1. Effects of acetone on survival of *Pratylenchus scribneri*.

Concentration (%)	Number of test vials	Mean # of survivors (%)	Range (%)
Control	8	100^{x}	68-127
2	8	64^{x}	38- 88
4	4	66	46- 76
6	8	63^{x}	45- 94
8	8	64^{x}	42- 84
9	4	65	32- 89
10^{z}	8	16^{x}	0- 41

^{x}represents mean of two experiments
$^{z}LC_{50}$

Table IV-2. Effects of methanol on survival of *Pratylenchus scribneri*.

Concentration (%)	Number of test vials	Mean # of survivors (%)	Range (%)
Control	4	100	74-142
2	4	90	65-113
6	4	81	54-129
8	4	85	69- 95
9	4	52	28- 82
10	4	61	44- 82

Table IV-3. Effects of N-acetyl loline on survival of *Pratylenchus scribneri*.

Concentration mM	**Number of test vials**	**Mean # of survivors (%)**	**Range (%)**
Control	9	100[y]	55-133
0.03	3	87	75-103
0.28	3	87	76-102
0.56	3	109	103-118
1.10	3	69	56- 85
2.20	6	124[x]	91-169
5.00	6	138[x]	97-180

[x]represents mean of two experiments
[y]represents mean of three experiments

Table IV-4. Effects of N-formyl loline on survival of *Pratylenchus scribneri*.

Concentration mM	**Number of test vials**	**Mean # of survivors (%)**	**Range (%)**
Control	6	100^x	75-124
0.1	3	108	95-128
0.5	6	90^x	79- 97
1.0	6	76^x	61- 85
2.0	6	76^x	65- 85
3.0	6	76^x	65- 87
5.0	3	74	71- 77

^{x}represents mean of two experiments

survival at 5 mM was 74 percent. These compounds were not tested at higher concentrations because they became insoluble. In concentrations above 1.0 mM, counting trays contained yellow droplets. This suggests poor solution of the alkaloids, indicating that results may not be valid. Future experiments will address this problem by use of different solvents, buffers, or more soluble salts of lolines.

In N-methyl loline dihydrochloride (Table IV-5), mean survival at 5 mM, the highest concentration tested, was 47 percent. In loline dihydrochloride (Table IV-6), mean percentage of survivors was 46 percent at 4.0 mM, the highest concentration tested. These results with dihydrochloride salts may be due to pH effects since pH of controls was not adjusted to that of test vials. This will be corrected in future experiments by use of buffers.

Ergot alkaloids. Survival rates were quite variable, with only 51 percent survival at 0.010 mM and higher survival rates at higher concentrations (Table IV-7). However, the ergotamine tartrate did not dissolve completely, which may explain discrepancies. These tests are inconclusive and will be repeated, using a more soluble salt of ergotamine. The tartrate ion had no effect on survival rates (Table IV-7).

Table IV-5. Effects of N-methyl loline dihydrochloride on survival of _Pratylenchus scribneri_.

Concentration mM	**Number of test vials**	**Mean # of survivors (%)**	**Range (%)**
Control	9	100[y]	78-124
0.00008	4	88	66-102
0.00020	4	116	101-132
0.00040	4	91	72-129
0.00080	4	91	67-113
0.00200	4	104	83-118
0.00400	4	85	62- 98
0.00750	4	124	103-164
0.00800	4	100	90-108
0.02000	3	97	78-120
0.08000	4	92	79-110
0.17000	4	93	74-128
0.33000	4	118	104-129
1.00000	3	77	53-105
5.00000	3	47	39- 59

[y]represents mean of three experiments

Table IV-6. Effects of loline dihydrochloride on survival of *Pratylenchus scribneri*.

Concentration mM	**Number of test vials**	**Mean # of survivors (%)**	**Range (%)**
Control	6	100[x]	55-133
0.1	3	89	70-104
0.5	3	75	57- 98
1.0	3	96	80-111
3.0	5	60[x]	44- 75
4.0[z]	6	46[x]	16- 64

[x]represents mean of two experiments
[z]LC_{50}

Table IV-7. Effects of ergotamine tartrate (E.T.) and tartaric acid (T.A.) on survival of *Pratylenchus scribneri*.

Conc. mM	Number of test vials		Mean # of survivors (%)		Range (%)	
	E.T.	T.A.	E.T.	T.A.	E.T.	T.A.
Control	3	3	100	100	91-116	91-116
0.010	3	3	51	108	28- 95	88-122
0.033	3	3	85	124	80- 89	116-129
0.100	3	3	52	106	42- 71	78-133
0.333	3	3	71	94	46- 98	90- 97

SUMMARY

A bioassay technique was developed to determine lethality of fescue alkaloids on plant-parasitic nematodes. Apparatus was designed to determine number of nematodes which survive exposure to dilutions of test compounds. Effective exposure times and inversion times were determined. Effects of different concentrations of common organic solvents on survival of nematodes were determined.

Results provide information on efficiency of the technique and approximate lethalities of tested compounds. Experiments will be repeated, using a wider range of effective concentrations which will determine LC_{50} levels accurately. Solubility of compounds will be determined. If compounds are not soluble at concentrations to be tested, more soluble salts or different solvents or buffers will be used. The comparative effects of controls with pH adjusted to that of test solutions by use of buffers will be determined. Number of replicates will be increased in order to decrease variance. Longer exposure times will be investigated.

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V. CONCLUSIONS

Populations of two species of plant-parasitic nematodes with different feeding patterns were lower on endophyte-infected fescue than on endophyte-free tall fescue. Pratylenchus scribneri feeds on cortex cells and M. graminis feeds on cells of the vascular system. This suggests presence of toxic substances in endophyte-infected tall fescue root systems.

Two species of nematodes did not show evidence of depressed numbers. Helicotylenchus pseudorobustus is an ectoparasite that feeds on endoderm, pericycle, and cortex cells, but does not remain in the root. An undescribed species of Meloidogyne which is parasitic on clover but not on tall fescue was not affected by the presence of endophyte-infected tall fescue roots in the rhizosphere.

A bioassay technique is described which can determine relative toxicities to nematodes of substances extracted from endophyte-infected fescue. Use of this bioassay can help in identification of toxins in the roots and determine their lethality to nematodes.

Higher levels of plant-parasitic nematode infestation may cause problems in establishment and maintenance of E- tall fescue pastures especially under drought conditions when root damage by nematodes can be a critical

factor in plant survival. Development of tall fescue infected with an endophyte which produces substances resulting in pest resistance but not harming livestock would be of great benefit to agriculture.

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APPENDIX

PROPAGATION OF PRATYLENCHUS SPP. ON FESCUE CALLUS

Callus tissue. 'Forager' and 'KY 31' tall fescue callus tissue (embryo-derived; Lowe and Conger 1979) was maintained on SH-30 medium (Schenk and Hildebrandt 1972) with 30 μM dicamba (3,6-dichloro-O-anisic acid, Velsicol Chemical Corp.), 30 g/l sucrose, 9 g/l Difco bacto-agar, and 1 g/l inositol, adjusted to pH 5.6 (modifications are as reported by Conger and McDaniel 1983, for growth of fescue callus tissue). Pieces (8 mm x 8 mm) of callus were placed on the medium in deep petri dishes, 100 mm x 20 mm. Callus was incubated in the dark at room temperature and subcultured at 8-wk intervals.

Nematodes. Inocula of *Pratylenchus scribneri*, *P. brachyurus* Filipjev & Stekhoven, and *P. agilis* Thorne & Malek were obtained from R. N. Huettel (Plant Protection Institute, USDA-ARS NER, Beltsville, MD 20705) on 'I. O. Chief' corn (*Zea mays* L.) root explants. Root explants were inspected at 35x magnification to determine infection with nematodes. Roots which contained nematodes were cut into 10-20 mm sections. Three sections, obtained from different plates, were placed on each of three pieces of callus tissue in a plate. Plates were sealed with Parafilm™ and placed in the dark at room temperature.

After three months, calli were inspected for infection at 35x magnification. Nematodes were visible in or near callus. Growth of highly infected callus tissue was less than that of uninfected controls. Sterile callus was routinely added to infected callus in order to provide adequate feeding sites. Infected callus was subcultured and placed on SH-30 at 8-wk intervals.

Eight weeks after subculturing, nematodes were extracted from callus by modified centrifugal-flotation (Jenkins 1964; Dunn 1972). Callus was placed in a Waring Blendor™ with 100 ml sucrose solution (454 g/l) and blended for two 15-sec intervals at high speed (approximately 12,600 rpm). Suspension was poured into 50-ml centrifuge tubes and centrifuged at 550 x g for 4 min. Supernatant was decanted onto a 500-mesh (25 μm pores) sieve. Nematodes were collected from the sieve and counted. Numbers of nematodes/callus piece ranged from 5000 to 10,000. Nematodes were also extracted from callus in a modified Seinhorst mist apparatus (Southey 1970). Callus was placed on Nitex™ nylon screen, with 23 μm pores (Tetko, Inc., 420 Saw Mill River Road, Elmsford, New York), glued between two 1-cm long pieces of 10-cm diameter PVC (polyvinyl chloride) pipe. This apparatus was placed in a funnel (10-cm diameter). The stem of the funnel was suspended in a 250-ml flask. A fine mist was

sprayed over the callus for 10 sec every 10 min for 2 wks. Nematodes emerged from the callus, washed through the screen, and were collected at 24-hr intervals from water in the flask. Numbers were highly variable and nematodes continued to emerge from callus for one month.

Determination of toxicity. Acetone and extracts of E+ and E- tall fescue roots were added to SH-30 media in plates to determine effect on nematode populations after 8 wks incubation. No nematodes survived in plates with added root extracts or in plates containing over 0.1 percent acetone.

VITA

Carol A. Kimmons was born in Nashville, Tennessee, in 1940. She graduated from Alamogordo High School in New Mexico in 1958. In 1962 she received a Bachelor of Science degree in Geology at the University of New Mexico, Albuquerque. She was a Peace Corps Volunteer in Nigeria and later taught science in India, Haiti, and Tennessee. In Dunlap, Tennessee, she worked in adult education and received two grants to write books for adult beginning readers.

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