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Abstract

The presence of the endophytic fungus *Acremonium coenophialum* Morgan-Jones et Gams in tall fescue *(Festuca arundinacea* Schreb.) induces toxicity when this grass is grazed by cattle; however, there is evidence that removing the endophyte reduces the stand vigor and longevity of fescue. A field trial was conducted to determine the effects of water supply and the presence of the endophytic fungus on plant growth, drought tolerance, and soil nematode populations in 'Kentucky 31' tall fescue. The design included two factors, level of endophyte infection (0 **and 75%) and** irrigation regime (none, low, and high). Where water deficits occurred, herbage yield and leaf area were lower, and percentage dead tissue and canopy minus air temperature were greater in endophyte-free compared with endophyte-infected rescue. Soil populations of *Pratylenchus scribneri* and *Tylenchorhynchus acutus* were substantially higher in the noninfected than in the endophyte-infected plots. The endophyte apparently confers drought tolerance to Kentucky 31 tall fescue, **and** this effect may be at least partially mediated through enhanced resistance to soil-borne nematodes.

Introduction

Tall fescue *(Festuca arundinacea* Schreb.) is a principal pasture grass in the southeastern **and** eastern midsection of the USA because of its high yield, persistence, pest resistance, and tolerance to environmental stress. However, there exists a set of disorders in animals grazing tall fescue, referred to collectively as fescue toxicosis, which results in low reproduction rates, milk production, and weight gain (Steudemann and Hoveland, 1988). The occurrence of fescue toxicity has been linked to the presence of an endophytic fungus *(Acremonium coenophialum* Morgan-Jones et Gams) that grows intercellularly in fescue and produces toxic alkaloids (Bacon *et al.,* 1986).

Replacing endophyte-infected pastures with fungus-free plantings can result in improved animal * Published with the approval of the Director of the Ark. Agric. Exp. Stn.

performance and health (Steudemann **and** Hoveland, 1988); however, evidence suggests that absence of the endophyte diminishes the vigor **and** stress tolerance of tall fescue. *Acremonium* spp. have been shown to enhance the seed germination rate, seedling vigor, and degree of seed set in tall fescue and perennial ryegrass *(Lolium perenne* L.) (Clay, 1987). Pedersen and Rodriguez-Kabana (1986) demonstrated that numbers of *Helicotylenchus* nematode species were depressed by at least 66% in soil containing endophyte-infected tall fescue compared with fungus-free fescue. Read **and** Camp (1986) observed severe stand depletion in drought-stressed tall fescue with no endophyte, whereas stands of infected fescue of the same cultivar were intact. Water supply was not controlled in their trial. Our objectives were to determine the influence of *A. coenophialum* infection on the response of tall fescue to a soil-water gradient and on soil nematode populations.

Materials and methods

Plots of'Kentucky 31' tall fescue, with and without the endophyte, were established on a fineloamy, siliceous, thermic Typic Hapludalf soil at Fayetteville, Arkansas, USA, by sowing into a prepared seedbed on 22 October 1986. During the spring and early summer of 1987, the plots were mowed twice, fertilized according to soil tests, and irrigated with 76 mm of water to promote vigorous, uniform establishment. At the beginning of the growth period under study (21 July), plots were again mowed to 7cm and fertilized with $45 \text{ kg N} \text{h} \text{a}^{-1}$ as ammonium nitrate. The experimental design was a strip-split plot with two factors, endophyte infection level (0 and 75%) and irrigation level (none, low, and high), arranged in eight replications. Endophyte level was measured by randomly sampling 20 5-cm tiller bases in each plot in early June, 1987. Leaf sheath sections were stained and microscopically examined according to Clark et al. (1983). Infection is expressed as a percentage frequency of hyphal detection in the tiller samples.

The irrigation levels were achieved by constructing a line-source sprinkler system between two rows of four replications each, thereby establishing a soil-water gradient from the irrigation line to 4.6 m into the plots. Those portions of the plots beyond 4.6 m received no water. The irrigation system consisted of Toro 12F nozzles on 1-m risers spaced 2.44m apart on 38-mm diameter PVC pipe. This riser spacing was determined to give the desired water distribution by using the model of Willardson et al. (1987). Irrigation frequency was designed to maintain soil-water pressure between -0.01 and

 -0.03 MPa at 0.15 to 0.18 m depth in the high water treatment (0.9 m from the sprinkler line). The location of the low-water treatment was approximately 3m from the sprinkler line and corresponded to where leaf rolling indicated the development of drought stress (soil water pressure between -0.06 and -0.08 MPa).

Canopy temperature was measured at 1500 h on 3 August with a hand-held infrared thermometer (Everest Interscience Inc., Tustin, California, USA). Three readings were taken per irrigation level for each plot, and canopy minus air temperature (T_c-T_a) was calculated. Ten days later, shoot dry matter yield of live leaf tissue and yield components were measured. Yield components consisted of specific leaf weight, leaf area per tiller, and percentage dead tissue. Four 20 -cm \times 20-cm quadrats were clipped to 3 cm and bulked across four locations per irrigation level in each of four replications for growth analysis and yield component analysis. All plots were harvested at the three irrigation levels on 17 August with a rotary mower to a 7-cm height for determining final herbage yield. Soil was sampled on 19 August to determine nematode populations by bulking 10 soil cores (15 cm deep by 2.5 cm diameter) across irrigation levels in each plot. After mixing thoroughly a 236ml aliquot was taken, and nematodes were extracted with the roiling-seiving-Baermann funnel technique (Christie and Perry, 1951). Population counts are expressed as the number of nematodes of all post-embryonic stages per 100ml of soil. Data for all measurements were analyzed by analysis of variance. The main effect of irrigation in this design was not a valid comparison (Hanks *et al.,* 1980).

Table 1. Effect ofendophyte presence averaged over irrigation levels on herbage dry matter yield, specific leaf weight, leaf area per tiller, dead tissue, canopy-air temperature (T_c-T_a) , and soil nematode populations

*** Means significantly different at $P < 0.05$, and 0.01, respectively.

Air temperature was 36.0° C.

Results and discussion

The final herbage yield accumulated over the 4-week growth period was significantly higher in plots with endophyte (Table 1); however, the advantage was apparent only in the nonirrigated and low-irrigation treatments where drought stress was evident (Figure la).

Selected components of the herbage were measured to determine how endophyte presence affected the response of fescue to water supply. The higher herbage yield of the infected fescue was not explained by an enhanced specific leaf weight; indeed, specific leaf weight tended to be lower $(P = 0.08)$ in the infected treatment (Table 1, Figure 1b). Leaf area per tiller, on the other hand, was significantly lower in the noninfected than in the endophyte-infected grass at all irrigation levels, with the differences more pronounced in the nonirrigated and low irrigation treatments (Figure lc). Many plants respond to drought stress by decreasing their functional leaf area to minimize transpiration loss (Begg, 1980). The endophyte-free fescue apparently underwent greater stress under limited water supply than the infected grass, causing enhanced leaf senescence. This plant response was demonstrated by the significantly higher percentage of dead leaf tissue (dry weight basis) in the endophyte-free plants when not irrigated (Figure ld).

Canopy temperature minus air temperature has been used as an indicator of the degree of drought stress in the plant (Jackson, 1982). Negative values (leaves cooler than the surrounding air) indicate well-watered, actively transpiring leaves, whereas positive values indicate stress-induced stomatal closure and reduced evaporative cooling of the leaves. Our results showed that as water supply decreased along the irrigation gradient, drought stress developed more severely in the noninfected than in the infected fescue (Figure le).

Soil populations of *Pratylenchus scribneri* Steiner and *Tylenchorhynchus acutus* Allen were substantially higher for endophyte-free than for infected fescue (Table 1). *P. scribneri* is a migratory endoparasite which has been shown to reduce growth of tall fescue (Minton, 1965); however, no information was found in the literature concerning the effects of *T. acutus,* an ectoparasite, on fescue.

Fig. 1. (A) Herbage dry matter yield, (B) specific leaf weight, (C) leaf area per tiller, (D) percentage dead tissue, and (E) canopy minus air temperature of tall fescue at 0% (----) and 75% (---) *Acremonium coenophialum* infection and at three levels of irrigation. LSD *bars* compare endophyte means within irrigation levels.

6 Endophyte effects on tallfescue growth

Our results provide further evidence that A. *coenophialum* **enhances the drought tolerance of its host, tall fescue. Over the duration of this preliminary field study, non-infected fescue stands exhibited greater leaf senescence, reduced green leaf area per tiller, and higher canopy temperature, indicating a reduced capacity to absorb and transpire water for the maintenance of active growth. Prolonged drought could lead to tiller mortality and stand depletion. The mechanism by which A.** *coenophialum* **improves host-plant water relations is not known. Although the fungus itself is generally not found in roots (Hinton and Bacon, 1985), allelochemicals synthesized in the endophyteinfected crowns and leaf sheaths may possibly be translocated to the roots and deter plant parasitic nematodes, thereby allowing greater root growth and more effective water extraction. These results, along with those of Clay (1987), indicate an important ecological role of** *A. coenophialum* **in contributing to the persistence and competitiveness of tall fescue under conditions of environmental and biotic stress. Furthermore, our data suggest that the increased drought tolerance in endophyte-infected fescue may be partially mediated through enhanced resistance to nematodes.**

References

Bacon C W, Lyons P C, Porter J K and Robbins J D 1986 Ergot toxicities from endophyte infected grasses. Agron. J. 78, 106- 116.

- Begg J E 1980 Morphological adaptations of leaves to **water** stress. *In* Adaptation of Plants to Water and High Temperature Stress. Eds. N C Turner and P J Kramer. pp 33-42. John Wiley and Sons, New York.
- Christie J R and Perry V G 1951 Removing nematodes from soil. Proc. Helminthol. Soc. Wash. 18, 106-108.
- Clark E M, White J F and Patterson R M 1983 Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of *in vitro* culture of **the** fungus. J. Microbiol. Meth. 1, 149-155.
- Clay K 1987 Effects of fungal endophytes on the seed and seedling biology of *Lolium perenne* and *Festuca arundinaeea.* Oecologia 73, 358-362.
- Hanks R J, Sisson D V, Hurst R L and Hubbart K G 1980 Statistical analysis of results from irrigation experiments using the line-source sprinkler system. Soil Soc. Am. J. 44, 886-888.
- 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,
- Jackson R D 1982 Canopy temperature and crop water stress. *In* Advances in Irrigation, Volume I. Ed. D Hillel. Academic Press, New York.
- Minton N A 1965 Reaction of white clover and five other crops to *Pratylenchus scribneri.* Plant Dis. Reptr. 49, 856-859.
- Pedersen J F and Rodriguez-Kabana R 1986 Response of nematodes to *Acremonium coenophialum* infected tall fescue. Agron. Abst. p. 120.
- Read J C and Camp B J 1986 The effect of the fungal endophyte *Aeremonium eoenophialum* in tall fescue on animal performance, toxicity, and stand maintenance. Agron. J. 78, 848- 850.
- Steudemann J A and Hoveland C S 1988 The fescue endophyte: History and impact on animal agriculture. J. Prod. Agric. 1, 39-44.
- Willardson L S, Oosterhuis D M and Johnson D A 1987 Sprinkler selection for line-source irrigation systems. Irrig. Sci. 8, 65-76.