

Occurrence of *Acremonium coenophialum* in Tall Fescue in Tennessee

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ABSTRACT

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Enzyme-linked immunosorbent assay was used for detection of *Acremonium coenophialum*, the endophyte associated with fescue toxicity in cattle. Thirty percent of the tall fescue (*Festuca arundinacea*) plants sampled throughout Tennessee contained the fungus. Plants with the fungus were found in 83% of the counties sampled. Dissemination of infested seed may account for the wide distribution.

Tall fescue (*Festuca arundinacea* Shreber) is an important pasture grass in the midsouthern United States because of its ease of establishment in various soils and its persistence in less than optimum climatic conditions. Tennessee has more than 1,420,000 ha of tall fescue pastures supporting livestock. Tall fescue has been associated with poor performance of cattle under certain conditions, despite its excellent nutritional qualities (4). The tall fescue toxicity syndrome in cattle is characterized by symptoms that include increased respiration rates, elevated body temperatures, excessive salivation, lameness, nervousness, and rough hair coats (1,5,6,8,9,16,17). In 1955, Merriman et al (10) reported fescue toxicity in Tennessee but were uncertain of the cause.

Acremonium coenophialum Morgan-Jones & Gams (formerly *Epichloe typhina* (Pers.) Tul. (13)), an endophytic colonizer of tall fescue, is believed to be associated with the toxicity syndrome in cattle (11). The fungus has been found in tall fescue plants in Tennessee and neighboring states (11). Siegel et al (15) found the endophyte in 97% of the fescue fields sampled in Kentucky.

Because of the economic importance of tall fescue, the occurrence of the endophyte in the state, and current reports of toxicity symptoms in cattle in Tennessee, a study was undertaken to determine the distribution of *A. coenophialum* in tall fescue pastures in Tennessee.

MATERIALS AND METHODS

Sampling procedure. Six fescue samples consisting of two plants from each of three locations were collected from each of 79 counties in Tennessee. Plants were collected from March 1982 to September 1983. Samples were planted in autoclaved soil in 15-cm pots in the greenhouse, watered, and fertilized. The plants were grown for a 2-wk acclimation period before culm samples were removed.

Four to six culm pieces were collected from each pot, placed in coin envelopes, and dried in an oven at 60 C for at least 2 days. After drying, sections were cut from each culm to yield a uniform sample length of 3 cm. The sample was pulverized to a dry powder with a mortar and pestle. Occasionally, 0.5 g of sterile, washed sand was added to assist grinding. Two milliliters of PBS-Tween-PVP buffer (ph 7.4, 0.02 M phosphate-buffered saline plus 0.05% Tween 20 plus 2% polyvinylpyrrolidone) was added to the powder and mixed to a fine slurry.

ELISA. The enzyme-linked immunosorbent assay (ELISA) described by Johnson et al (7) was used. Cultures of *A. coenophialum* used to produce antigen were obtained from T. P. Pirone, University of Kentucky, Lexington. The mycelium was cultured in M43 liquid medium following the procedure of Bacon et al (3). The antigen was partially purified and antiserum was produced by subcutaneous injections into a rabbit. Rabbit serum was extracted, partially purified, and conjugated with alkaline phosphatase (Type VII-S, Sigma, St. Louis, MO).

Dilution series consisting of positive and negative tall fescue tissue controls and freeze-dried mycelium at 10 and 1 mg/ml and 100 µg/ml were prepared to determine the concentration at which *A. coenophialum* could be detected in fescue

tissue. Another dilution series was made to confirm that the preimmune serum would not react with the fescue tissue in the ELISA analysis. Positive and negative fescue tissue controls were established by observing the presence or absence of the fungus in fescue tissue by microscopic examination. *A. coenophialum* mycelium was removed from the M43 liquid culture, washed in distilled water, frozen, and dried for use as a positive mycelium control. Samples were arranged in a completely randomized design in the ELISA plate with six replicates of each of the three control dilutions and six PBS-Tween-PVP buffer controls. Absorbance values were measured at 405 nm with a Dynatech ELISA reader (Alexandria, VA). No periphery plate wells were used.

Preimmune rabbit serum was purified and enzyme-conjugated following the same procedure as that for the antiserum. The dilution series used for the known fescue control was followed for the preimmune rabbit serum, since those dilutions had previously been found to have a positive reaction. Dilutions more than 100 µg/ml in previous tests were found to be indistinguishable from the readings of the PBS-Tween-PVP buffer control, and therefore, these dilutions were not used.

A. coenophialum mycelium was examined microscopically after staining with aniline blue in lactic acid following the procedure of Bacon (2) and was compared with previously published plates by Bacon et al (3) and Neill (12) and sketches of the endophyte by Morgan-Jones and Gams (11).

RESULTS AND DISCUSSION

Absorbance readings were taken for the reaction of the preimmune rabbit serum with the fungus-infected fescue tissue, the fungus-free fescue tissue, the freeze-dried mycelium, and the PBS-Tween-PVP buffer control. There were no differences in any of these readings, indicating that the positive reactions in the ELISA were due to antibody production by the rabbit specifically for *A. coenophialum*.

Positive fescue tissue control readings were 0.60, similar to the freeze-dried mycelium control reading of 0.58. The negative fescue control reading was 0.23,

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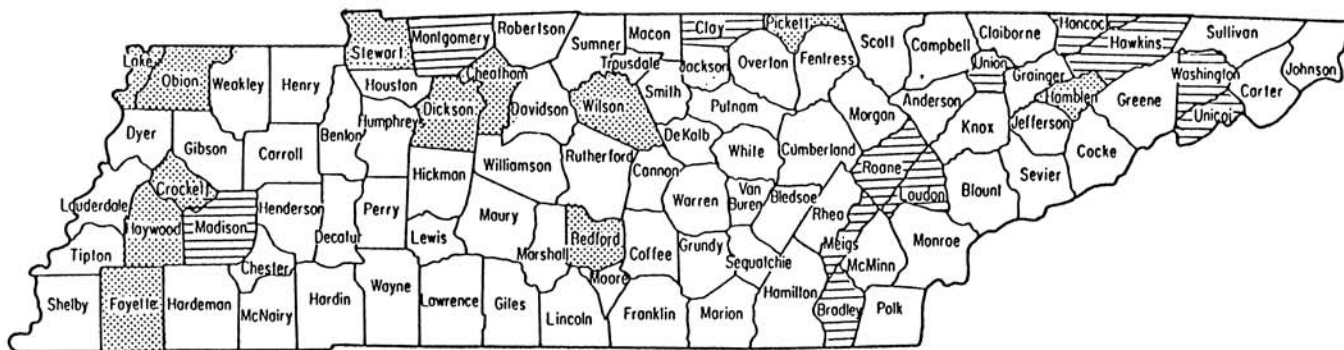


Fig. 1. Counties sampled for presence of *Acremonium coenophialum* in Tennessee: ▨ = no samples received, ▩ = samples negative, and □ = at least one of six samples gave a positive reaction for the fungus. (From *Tennessee Farm & Home Science*, No. 131, 1984. Reproduced by permission.)

Table 1. Distribution of *Acremonium coenophialum* in *Festuca arundinacea* in Tennessee

Agricultural extension district	Total samples (no.)	Positive samples (no.)	Positive samples (%)	Counties participating (no.)	Counties with fungus ^a (no.)	Counties participating (%)
1	110	25	23	20	15	95
2	123	31	25	21	16	96
3	66	25	38	11	11	69
4	78	25	32	13	12	87
5	76	30	40	14	13	67
Totals	453	136	30	79	67	83

^aCounties that contained at least one positive sample of six submitted from each county.

slightly less than the PBS-Tween-PVP buffer control reading of 0.27. A baseline rejection point for fescue samples in which fungus presence was unknown was established for each ELISA plate. This point was twice the mean of the negative fescue control readings for each plate.

A. coenophialum was found in at least one of six samples from each county (Fig. 1). Distribution data are presented in Table 1. One hundred thirty-six of the 453 samples examined, representing 83% of the counties sampled, contained positive levels of *A. coenophialum*. Although the antiserum sensitivity was only in the range of 0.1–1 mg/ml in the antiserum available, 30% of the tall fescue samples received from throughout Tennessee had detectable levels of *A. coenophialum*. The microscopic comparison of *A. coenophialum* with previously published descriptions confirmed that the endophyte present in Tennessee tall fescue samples was morphologically similar to the endophyte that has been linked to fescue toxicity.

The endophyte is widely distributed in tall fescue in Tennessee, and increased sampling may indicate greater distribu-

tion in individual counties. The number of fescue samples in this study with detectable levels of *A. coenophialum* may be unrealistically low, because the titer of the antiserum was low. It is believed that low concentrations of the endophyte in some samples may remain undetected.

It has been demonstrated that *A. coenophialum* is seedborne (14). No other means of dissemination or invasion by the fungus have been elucidated. It can only be speculated that the present wide distribution is due to the transporting of infested seed.

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