Incidence and Distribution of the Tall Fescue Endophyte in the United States

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ABSTRACT

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Levels of the endophytic fungus (*Acremonium coenophialum*) were determined in samples of tall fescue (*Festuca arundinacaea*) plants and seed over a 3-yr period. The mean infection of plant samples from 26 states was 58%; however, seed samples were less infected (54%), particularly when live endophyte was assayed (6%). The cultivar KY-31 was most heavily infected, although endophyte-free seed of this and other cultivars were common. Livestock owners reporting symptoms of fescue toxicity also reported higher levels of infection than owners not observing symptoms.

Additional key words: agalactia, *Epichloë typhina*, fescue foot, summer syndrome

Bacon et al (2) first made the association between the presence of an endophytic fungus in tall fescue (*Festuca arundinacaea* Schreb.) and symptoms in grazing animals known generally as fescue toxicity. Subsequent investigations confirmed the presence of *Acremonium coenophialum* Morgan-Jones & Gams in tall fescue seed and pastures (4,8,11) and described specific physiological effects on animals fed endophyte-infected (EI) fescue or extracts from it (5,6,9,10,15). Other species of endophytic fungi are widely distributed in the Poaceae and Cyperaceae, producing similar toxic effects in grazing animals (2,12,13).

The implication of *A. coenophialum* as the causal agent of fescue toxicity has stimulated interest among seedsmen and livestock producers to test seed and pastures for potentially damaging levels of the endophyte. In June 1983, we began soliciting samples of tall fescue from all interested sectors of the forage industry. In the following 3 yr, 4,645 such samples were analyzed. A brief questionnaire accompanying samples provided information such as location, cultivar, animal species, breed, and specific livestock problems. The data were summarized to determine if any relationship exists between levels of the endophyte and any of these parameters.

MATERIALS AND METHODS

Sampling. Samples were submitted to the Auburn University Fescue Diagnostic Center by seedsmen, county extension agents, consultants, cattle producers, and owners of various other livestock. Not reported are samples from foreign countries or research samples that may have been treated with fungicides or that were deliberately selected to establish experimental pastures of high or low fungal infestation. The remaining 2,405 samples were considered representative of cultivated tall fescue in the United States. Specific instructions for sampling and mailing were provided in a brochure, available from the Fescue Diagnostic Center, and only samples judged to have been taken properly were processed. Pastures were sampled by taking single tillers from plants randomly distributed throughout the field. The lowest 10 cm of 30 tillers (without roots) were combined to constitute a sample, placed in damp paper toweling in a plastic bag, and mailed directly to the laboratory. Clients were advised to not let a single sample represent more than 12 ha and to not mix pastures known to have been seeded at different times or with different seed lots. Upon receipt, samples were inspected for contaminating saprophytic fungi, the presence of grasses other than tall fescue, and general acceptability. Clients were advised to resample in certain instances. Samples were frozen until the staining procedure could be conducted.

Sampling instructions for seed were as follows: 50-g samples were to represent no more than 908 kg. Clients were advised to probe seed in bulk to obtain a random sample. With bagged seed, the recommendation was to sample the first five bags of a lot plus 10% of the remaining bags.

Detecting the endophyte. Various methods of detecting the endophyte have been reported (3,7). Early in the survey, we evaluated them for accuracy and efficiency, eventually adopting the following methods.

Thirty tillers per sample were selected at random for the staining procedure. Because the endophyte appeared to be concentrated in the base of the leaf sheath (4), this portion of the plant was examined microscopically. The basal portion of the sheath of the outermost intact leaf was removed, and a section 3–5 mm wide and 10 mm long was isolated. With a scalpel, the upper and lower epidermis were separated and placed mesophyll-up on a microscope slide. Sections were flooded with aniline blue-lactic acid (1 g of aniline blue + 100 ml of water + 200 ml of 85% lactic acid) for 1 min and blotted dry. Sections were then mounted in water and examined at 200×. Tillers with any identifiable *A. coenophialum* hyphae were scored as positive. Several criteria were used to distinguish *A. coenophialum* from other fungi in situ. Hyphae never invade cells of the tall fescue host, seldom branch, and have a typically serpentine appearance in older host tissue.

Seed were digested in 5% (w/v) NaOH at room temperature for 16 hr and rinsed in running tap water for 3 min. Pericarps were removed with forceps, and seed were macerated in aniline blue-lactic acid stain. Fifty seeds were crushed under no. 2 coverslips and individually examined at 200×. As with the plant samples, seed with any identifiable hyphae of *A. coenophialum* were scored as positive. Hyphae of *A. coenophialum* are typically found between the cells of the aleurone layer of the seed where serpentine hyphae often form dense mycelial mats.

Seed more than 6 mo old were subjected to the grow-out test procedure. Because the endophyte is known to decline in viability as the seed ages (15), determination of viable endophyte level requires examination of plants grown from seed. Seed were planted in 15-cm plastic pots, 50 seeds per pot × three pots per seed sample, in a peat/soil potting mixture with 3 g of 14-14-14 slow-release fertilizer. Plants were maintained in a greenhouse equipped with evaporative cooling (maximum 30 C) for 8 wk. Thirty plants of each seed lot were then subjected to the previously described diagnostic procedure. The mean age of seed tested by the grow-out test was about 1 yr but varied from 6 mo to 2 yr.

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RESULTS AND DISCUSSION

Distribution by state. Endophyte levels of samples are summarized by states in Table 1. During the survey, samples were received from 26 states. Many states were represented by inadequate sample sizes (Minnesota, New Mexico, New York, Texas, Utah, Washington, and West Virginia). Other states were part of a deliberate survey, and we believe additional sampling in these states would not result in an appreciable change in percentages (Alabama, Missouri, North Carolina, South Carolina, and Virginia). Mean infection levels were 58, 54, and 6% for plant stain, seed stain, and grow-out tests, respectively. In those states represented by at least 10 samples, infection levels varied from a low of 17% (Oregon) to a high of 73% (Maryland). When grouped by regions, the western states (California, New Mexico, Oregon, Utah, and Washington) were decidedly lower than the overall mean. We believe this difference is due to the predominance of cultivars other than KY-31 in these samples. Seed stain percentages were generally equivalent to plant stain percentages, but seed grow-out tests were dramatically lower in percent live endophyte. The geographic distribution of *A. coenophialum* in plant samples suggests that no edaphic or climatic limitations restrict its growth in any fescue population. Similarly, no reports exist of any level of genetic resistance in fescue to infestation by endophytic fungi.

Distribution by sample percentage. Percentage distributions for plants and seed tested by the staining method (Figs. 1 and 2) were similar and bimodal, with populations tending to have either very high or very low infection levels. Moderate infections were less frequent. When tested for viability by the grow-out test (Fig. 2), endophyte-free (EF) seed lots made up 68% of all such tests, and only eight of 302 grow-outs tested greater than 50% live endophyte. We believe that producer-submitted plant samples more nearly reflect the endophyte status of U.S. tall fescue pastures. On the other hand, the seed samples reported here do not reflect the status of existing pastures or the status of seed in commercial channels. Seedsmen are apparently sending mostly EF seed for testing, owing to the enhanced market value of seed that prove to be noninfected. Because endophyte levels in pastures and seed fields are relatively stable (15), there is little reason to test seed from sources known to be infected. This interest in EF seed is apparent when sample percentages are tabulated yearly. In 3 yr of testing, the percentage of EF pastures increased only

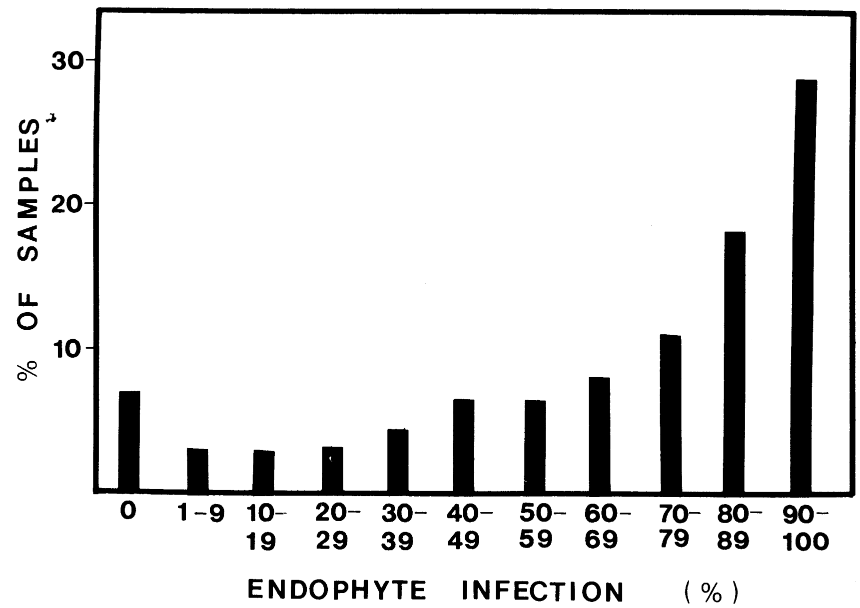

Fig. 1. Infection by *Acremonium coenophialum* in 1,483 tall fescue plant samples.

Table 1. Infection of tall fescue in the United States by *Acremonium coenophialum* (summary of infection levels of plant and seed samples from 1983–1986)

	Type of sample and detection test							
	Plant stain		Seed stain		Seed grow-out		Total	
State	*n*[a]	%	*n*	%	*n*	%	*n*	%
Alabama	342	59.23	247	51.05	79	2.47	668	49.49
Arkansas	44	62.30	6	55.17	16	8.88	66	48.70
California	4	15.50	3	15.33	···	···	7	15.43
Georgia	54	67.26	11	59.18	47	4.45	112	40.11
Illinois	21	51.71	···	···	1	0.00	22	49.36
Indiana	14	46.21	···	···	18	10.61	32	26.19
Kansas	33	53.27	9	34.78	3	0.00	45	46.02
Kentucky	25	48.64	5	41.80	2	0.00	32	44.53
Louisiana	13	45.00	···	···	···	···	13	45.00
Maryland	18	72.56	···	···	···	···	18	72.56
Minnesota	···	···	1	0.00	···	···	1	0.00
Missouri	33	57.45	211	62.83	90	4.80	334	46.66
Mississippi	3	62.00	15	62.93	···	···	18	62.78
North Carolina	368	52.99	16	39.69	4	0.00	388	51.90
New Mexico	5	41.00	···	···	···	···	5	41.00
New York	···	···	···	···	1	0.00	1	0.00
Ohio	29	50.10	···	···	···	···	29	50.10
Oklahoma	7	76.29	35	63.74	1	0.00	43	64.30
Oregon	···	···	42	10.81	8	48.63	50	16.86
South Carolina	257	66.72	4	51.25	14	0.29	275	63.11
Tennessee	56	62.11	8	38.13	8	16.75	72	54.40
Texas	1	76.00	2	82.00	1	0.00	4	60.00
Utah	1	0.00	···	···	···	···	1	0.00
Virginia	149	57.88	5	28.40	9	12.11	163	54.45
Washington	2	26.50	···	···	···	···	2	26.50
West Virginia	4	67.00	···	···	···	···	4	67.00
Total	1,483	58.47	620	54.21	302	5.98	2,405	50.32

[a] Number of samples tested.

slightly (5–7%, Table 2), whereas the percentage of EF seed lots as indicated by the grow-out test increased dramatically (44–82%, Table 2). Given the economic incentive of enhanced sales and a premium on EF seed coupled with newly enacted seed labeling regulations in several states, there has been an obvious increase in production of EF seed.

To test the efficacy of seed aging as an endophyte control method, we subjected seed at least 1 yr old to a sample-by-sample comparison by both the stain and grow-out procedures. For a period of 9 mo, from January to October 1984, all seed to be tested by the grow-out test was first tested by the stain test to determine percent endophyte at harvest. During this period, 72 seed lots that had been stored under unspecified warehouse conditions were tested from six states (Alabama, Georgia, Kansas, Missouri, Oklahoma, and Virginia). Stain tests on these seeds ranged from 21 to 100% infection, with a mean of 67.6%. Grow-out tests ranged from 0 to 23% live endophyte, with a mean of 4.6%. Twenty-seven of 72 seed lots were at least 5% infected. These data indicate that storage of infected seed has proven to be a successful nonchemical seed treatment for control of this pest but point out that uncontrolled storage does not always result in complete death of the endophyte and that testing by the grow-out test is necessary to monitor viability in storage.

In addition to infected seed that has been aged, we have tested EF seed produced by two other methods: 1) existing stands of KY-31 that were historically EF for unknown reasons and 2) other cultivars for which seed production has been oriented toward EF status, including repeated testing at several steps in production to prevent contamination by *A. coenophialum*. These have been essentially EF from breeders' seed and maintained so to enhance marketability.

Cultivars. Seventeen cultivars were identified in samples tested, but only 31% of samples were identified by cultivar (Table 3). In the eastern fescue-growing region, from which most of the samples came, it would be safe to assume that most of the remaining 69% of the samples were the KY-31 cultivar or progeny lines from old stands of KY-31. This is borne out by the similarity in percent infection of unknown and KY-31 classes (Table 3). Although other known cultivars made up only 3.5% of the samples tested ($n = 84$), infection levels are dramatically lower in every instance. Many of the non-KY-31 cultivars were low-endophyte cultivars such as Forager, Johnstone, Kenhy, MO-96, and Triumph. Clearly, KY-31 has a higher level of infection than most other cultivars.

Animal performance. Clients submitting pasture samples were asked to rate their animals' performance subjectively (good/fair/poor) and report any specific livestock problems in an effort to detect possible species/breed/endophyte interactions. In cattle, the largest species category ($n = 1,045$), breeds did not differ in their responses to endophyte levels. It was clear from the responses that some individual cattlemen reporting significant infestations have not noticed livestock problems. Conversely, some individuals with EF pastures have experienced problems that they have erroneously attributed to fescue toxicity. For all breeds combined, however, the mean infestation of the "good" performance category was 44%, whereas the "poor" category was 64% (LSD 0.05 = 8.5%). This implies that the effects of endophyte infestation produce symptoms that can be identified by the average cattleman, but subjective evaluations of clients were not always reliable indicators of endophyte levels. The same trends were generally evident for horses and other livestock species, but

Table 2. Percentage of U.S. tall fescue plant and seed samples determined to be endophyte-free (0% infection) by staining or grow-out tests

Year	Plant stain		New seed[a]		Old seed[b]	
	n[c]	%	*n*	%	*n*	%
1983	158	4.43	384	13.02	16	43.75
1984	670	6.87	147	17.01	102	50.98
1985	605	6.78	89	24.72	184	81.52
Total	1,433	6.56	620	15.65	302	69.21

[a] Percent determined by stain test on seed 6 mo old or less.
[b] Percent live endophyte determined by grow-out test on seed older than 6 mo.
[c] Number of samples tested.

Table 3. Percent infection of tall fescue cultivars caused by *Acremonium coenophialum*

Tall fescue cultivar	Type of sample and detection test							
	Plant stain		Seed stain		Seed grow-out		Total	
	n[a]	%	*n*	%	*n*	%	*n*	%
KY-31	260	63.60	246	58.48	160	5.54	666	47.59
Unknown[b]	1,217	57.64	322	54.67	116	3.06	1,655	53.24
Others[c]	6	25.59	52	9.70	26	21.72	84	11.00
Total	1,483	58.47	620	52.41	302	5.98	2,405	50.32

[a] Sample size.
[b] Probably represent KY-31.
[c] Represents 16 cultivars.

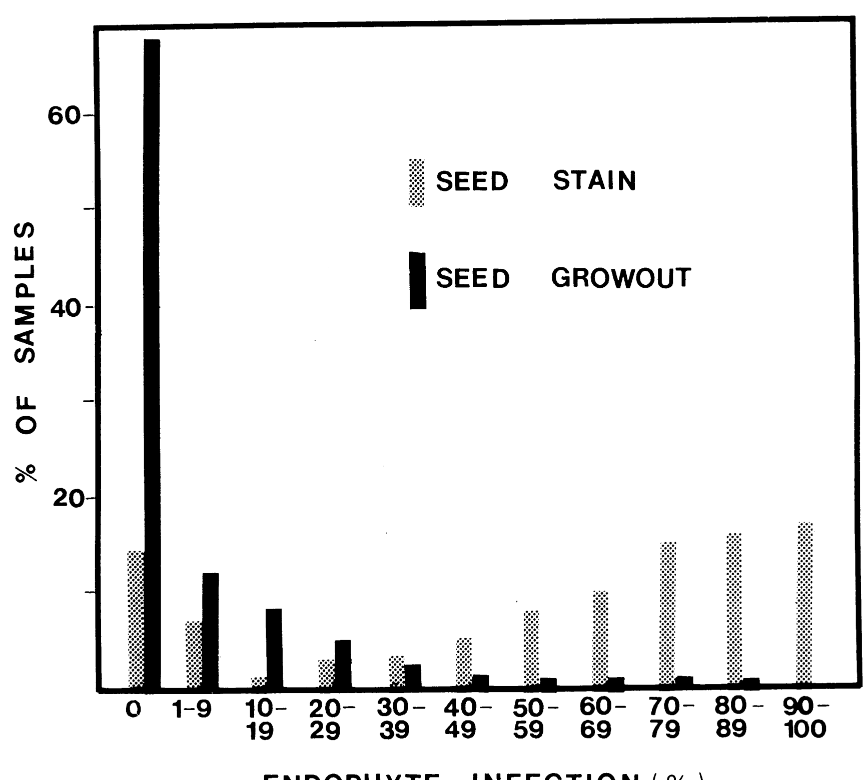

Fig. 2. Infection by *Acremonium coenophialum* in 620 seed samples tested by seed staining (318 samples) and the grow-out procedure for live endophyte (302 samples).

sample populations were too small for accurate evaluation.

Using the previously published regression of average daily gain (ADG) vs. percent infestation (15) and our mean for pasture samples of 58% as an estimate of infestation levels, the average U.S. cattle producer who grazes cattle on fescue is losing 0.63 kg ADG to fescue toxicity. This is in addition to breeding losses and other manifestations of the fescue toxicity syndrome.

Increased awareness of the effects of endophytic fungi is affecting all sectors of the forage industry. Livestock owners have begun replanting infested pastures, which in turn has created a demand for EF seed. Seedsmen have responded by increasing stocks of EF seed, and state regulatory agencies have begun testing and enforcing regulations regarding the advertising and sale of EF and EI seed. Because of the insidious nature of the endophyte, however, toxic fescue pastures will probably be present for the foreseeable future.

LITERATURE CITED

1. 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.
2. Bacon, C. W., Porter, J. K., Robbins, J. D., and Luttrell, E. S. 1977. *Epichloë typhina* from toxic tall fescue grasses. Appl. Environ. Microbiol. 34:576-581.
3. 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. Methods 1:149-155.
4. Hinton, D. M., and Bacon, C. W. 1984. The distribution and ultrastructure of the endophyte of tall fescue. Can. J. Bot. 63:36-42.
5. Hoveland, C. S., Haaland, R. L., King, C. C., Jr., Anthony, W. B., Clark, E. M., McGuire, J. A., and Holliman, J. L. 1980. Association of *Epichloë typhina* fungus and steer performance on tall fescue pasture. Agron. J. 72:1064-1065.
6. Hoveland, C. S., Schmidt, S. P., King, C. C., Jr., Odom, J. W., Clark, E. M., McGuire, J. A., Smith, L. A., Grimes, H. W., and Holliman, J. L. 1983. Steer performance and association of *Acremonium coenophialum* fungal endophyte on tall fescue pasture. Agron. J. 75:821-824.
7. Johnson, M. C., Pirone, T. P., Siegel, M. R., and Varney, D. R. 1982. Detection of *Epichloë typhina* in tall fescue by means of enzyme-linked immunosorbent assay. Phytopathology 72:647-650.
8. Long, E. A. 1984. Occurrence of *Acremonium coenophialum* in tall fescue in Tennessee. Plant Dis. 69:467-468.
9. Neal, W. D., and Schmidt, S. P. 1985. Effects of feeding Kentucky-31 tall fescue seed infected with *Acremonium coenophialum* to laboratory rats. J. Anim. Sci. 61(3):603-611.
10. Porter, J. K., Robbins, J. D., Norred, W. P., Garner, G. B., and Bacon, C. W. 1985. Serum prolactin and brain catecholamine metabolite depression in rats administered extracts of endophyte-infected fescue. J. Agric. Food Chem. 33:34-36.
11. Schmidt, S. P., Hoveland, C. S., Clark, E. M., Smith, L. A., Grimes, H. W., and Holliman, J. L. 1982. Association of an endophytic fungus with fescue seed or hay. J. Anim. Sci. 55:1259-1263.
12. Siegel, M. R., Latch, G. C. M., and Johnson, M. C. 1985. *Acremonium* fungal endophytes of tall fescue and perennial ryegrass: Significance and control. Plant Dis. 69:179-183.
13. White, J. F., and Cole, G. T. 1985. Endophyte-host associations in forage grasses. I. Distribution of fungal endophytes in some species of *Lolium* and *Festuca*. Mycologia 77(2):323-327.
14. Williams, M. J., Backman, P. A., Clark, E. M., and White, J. F. 1984. Seed treatments for control of the tall fescue endophyte. Plant Dis. 68:49-52.
15. Williams, M. J., Backman, P. A., Crawford, M. A., Schmidt, S. P., and King, C. C., Jr. 1984. Chemical control of the tall fescue endophyte and its relationship to cattle performance. N.Z. J. Exp. Agric. 12:165-171.