Seed Treatments for Control of the Tall Fescue Endophyte *Acremonium coenophialum*

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ABSTRACT

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The fungal endophyte *Acremonium coenophialum* has been implicated in fescue toxicosis, an animal disorder common on the 14×10^6 ha of tall fescue (*Festuca arundinacea*) grown in the United States. Nonchemical and chemical seed treatments were evaluated for their ability to eradicate the endophyte from infected fescue seed, the only known means of endophyte transmission. On-farm storage for about 12 mo or a hot-water treatment (presoak 6 hr at 5 C plus 20 min at 60 C) eradicated the endophyte in the seed tested. The sterol inhibitor triadimenol and its analog triadimefon were the most effective fungicides at the rates tested. Either chemical at 4.8 g a.i./kg seed reduced the viable endophyte level from 70 to 0% in the greenhouse with seed treated with a commercial seed treater 3 mo postharvest. Effects of the fungicides on seed germination varied with formulation and rate but in most cases were not significant ($P = 0.05$) by final germination count. In field plantings of seed treated with a commercial seed treater using triadimenol or triadimefon at 4.8 g a.i./kg seed, control ranged from 50 to almost 100%; variations in control were possibly due to soil differences among planting locations.

Additional key words: *Epichloë typhina*

Tall fescue (*Festuca arundinacea* Schreb.) is one of the most important cultivated forage species in the United States, with more than 14×10^6 ha in production (3). Tall fescue, a cool-season perennial grass, is widely grown but is best adapted to the transition zone between the northern and southern regions of the United States, where large numbers of livestock primarily graze one cultivar, Kentucky-31 (Ky-31). Although the forage quality of tall fescue is chemically similar to other perennial grass species grown in the Southeast, performance (conception rates, milk production, and weight gain) of animals grazing tall fescue is disappointingly lower than that of animals grazing other cool-season perennial grasses or fescue-clover mixtures (2–4,11,13,14). Poor performance on tall fescue has been associated with several animal disorders that are not fully understood in spite of extensive research (3).

The most common animal disorder associated with tall fescue is characterized by poor weight gain, reduced conception rate, retained winter hair coat, elevated body temperature, and increased respiration rate and is described by many terms, including poor animal performance, summer syndrome, and fescue toxicosis. Nationwide, severe economic losses result from reduced average daily gains (ADG) in the summer, which are frequently less than half those recorded in late spring or early fall (11).

In 1977, Bacon et al (1) demonstrated a high correlation between fescue toxicosis and presence of an endophytic fungus they identified as *Epichloë typhina* (Pers.) Tul. Now identified as *Acremonium coenophialum* Morgan-Jones & Gams (10), the tall fescue endophyte has been found in almost all Ky-31 tall fescue tested in the United States. In replicated grazing trials, endophyte-free pastures produced >50% higher ADG than endophyte-infested pastures (6,7). Infested pastures treated with fungicides produced ADGs equal to uninfested pastures and were almost 50% better than the infested control (M. J. Williams, *unpublished*). Although a strong association has been demonstrated, Koch's postulates have not been fulfilled for *A. coenophialum*, and as yet, a toxin(s) has not been identified.

There are no visible signs or symptoms of the fungus on tall fescue, precluding easy identification of infected plants (1,12). Spread of the endophyte from one area to another appears limited to movement via infected seed (12). Endophyte levels have not increased in 8 yr in an uninfested pasture separated from a heavily infested pasture only by a fence (E. M. Clark, *unpublished*). The fescue endophyte was reported to die in seed stored more than 12 mo (12); the uninfested tall fescue pastures used in the grazing studies of Hoveland et al (7) were presumably established from old seed. In preliminary efficacy trials, certain chemicals have controlled *A. coenophialum* (16). The objective of this research was to evaluate nonchemical treatments and selected fungicides for controlling *A. coenophialum* in fescue seed.

MATERIALS AND METHODS

Seed sources. Two lots of Ky-31 tall fescue seed were obtained in the fall of 1981, about 3 mo after harvest. One was produced by a certified seed producer in northern Alabama; the other was from a known infested field from the Black Belt Substation, Marion Junction, in west central Alabama. Both seed lots were used for experimental seed treatment test 1 (EST-1), whereas only the former was used for EST-2 and the nonchemical control test. Additional seed was obtained in 1982 from the same commercial producer and used in subsequent seed-treatment tests.

Endophyte evaluation. Incidence of the endophyte in all seed lots was determined using a digestion staining technique. About 10 ml of fescue seed was added to 50 ml of hot (60 C), concentrated HNO_3 (15.4 M) and stirred for 1 min. Digestion was stopped by adding 3–4 volumes of tap water to the acid-seed mixture, pouring immediately over a nylon screen, and rinsing several times with tap water followed by soaking for 2–3 min in tap water to soften. Individual seeds were placed on a microscope slide, flooded with a 1:5 aqueous dilution of a 1% aqueous analine blue:85% lactic acid (2:1) stain, and squashed with even downward pressure applied to the entire coverslip until the seeds were two to three times their original diameter. Squashed mounts were examined for the presence of the endophyte, as evidenced by extracellular convoluted, blue-stained hyphal fragments around the periphery of the squash interspersed with the densely stained aleurone cells. Percent infection was based on the examination of 50–100 seeds from each lot.

Incidence of viable endophyte in seeds in each treatment was determined by planting about 20 seeds in 11-cm-diameter pots in sterile potting soil. Pots were arranged in a randomized block

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design with five replicates in a cool greenhouse (25/15 C, day/night). Sixty to 70 days after planting, five seedlings from each pot were severed at soil level and frozen until they could be examined cytologically. The outer leaf of each seedling was removed and the epidermal surfaces of the lower centimeter of the leaf were separated by slicing through the mesophyll tissue with a scalpel. Both surfaces were mounted mesophyll up, stained with full-strength 1% aqueous aniline blue:85% lactic acid (2:1), and examined for presence of the fescue endophyte, which appeared as dark blue, septate (often corkscrew-shaped) hyphae running parallel to the mesophyll cell walls (Fig. 1). Samples were considered positive if any of the characteristic mycelium was found.

Germination. Germination was evaluated by placing 50 seeds from each treatment on a premoistened filter paper in petri dishes arranged in a seed germinator (8-hr light at 30 C alternating with 16-hr dark at 20 C) in a randomized block design with five replicates for 14 days. Germinated seeds were counted at 5 and 14 days, except EST-1 and EST-4, which were made at 14 days only. Seeds were considered germinated if the radicle was at least the length of the long axis of the seed.

Nonchemical control. To determine survival of the endophyte under farm-storage conditions, seed was obtained and planted at 3, 6, 8, and 12 mo postharvest as described previously. The seed had been stored in northern Alabama in an enclosed barn in 22.7-kg sacks stacked 2–3 m high on pallets. During the July 1981 to July 1982 storage period, no attempt was made to alter normal temperature and humidity fluctuations. Incidence of the viable endophyte in the seed lot after each storage period was determined as described and compared with original seed-infection levels determined by the seed-squash technique.

Seed stored for 3 mo on the farm was subjected to a hot-water soak (55 C) for 10 min with and without a 6-hr cold-water presoak (5 C). After treatment, seed was dried for about 2 hr at 30 C in a forced-air oven. Four months later, treatments were repeated using the same seed lot stored an additional 4 mo at room temperature (24 C) with the hot-water treatment time increased to 20 min. Germination and incidence of viable endophyte were determined for each treatment and for their respective controls.

Chemical control. All seed chemicals were applied to 45 g of seed with 9% (water:seed) aqueous carrier (4 ml) using a Gustafson Batch Lab Treater (Gustafson, Inc., Dallas, TX) except where noted. Treated seed were allowed to air-dry. Germination tests and greenhouse plantings were initiated within 24 hr of treatment.

In EST-1, both lots of 3-mo-old 1981 seed were treated with 1) triadimenol 150 FS (150 g a.i./L), 0.3 g a.i./kg seed (Baytan 150 FS); 2) triadimefon 50WP, 4.8 g a.i./kg seed (Bayleton 50WP); 3) CGA-64251 0.846E (100 g a.i./L), 0.1 g a.i./kg seed (Vangard); 4) metalaxyl 2E (240 g a.i./L), 0.3 g a.i./kg seed (Apron 2E); and 5) carboxin + thiram 200F (200 + 200 g a.i./L), 0.5 g + 0.05 g a.i./kg seed (Vitavax 200).

In EST-2, an additional formulation of triadimenol 2ST (240 g a.i./L) was used on 1981 commercially produced seed. Triadimefon was applied at the same rate as the triadimenol, but the carboxin + thiram rate was doubled from the previous test.

For EST-3, 1982 seed was treated earlier than 1 mo postharvest with triadimenol 150F (150 g a.i./L) at 1.2, 2.4, 3.6, and 4.8 g a.i./kg seed. Triadimefon 50WP was applied at the same rates plus a 9.6-g a.i./kg seed rate. Endophyte incidence in the seedlings was not determined until 4 mo after planting because seed dormancy slowed seedling emergence. To reduce dormancy, the 1982 seed was stored at room temperature for 3 mo and triadimefon treatments were reapplied (EST-4) with the addition of a 7.2- and 12-g a.i./kg seed rate.

Commercial quantities of 4-mo-old farm-stored seed were treated with a Gustafson Film Coater (Gustafson, Inc., Dallas, TX) with triadimenol 30F (33% a.i./L) and triadimefon 50WP at 4.8 g a.i./kg seed in 4% (water:seed) carrier. A commercial seed dye was included in each treatment and in the water-treated control to facilitate evaluation of coverage. Subsamples from each treatment and the control were sorted according to light, medium, and dark dye coverage and the effects on seed germination and incidence of viable endophyte were determined separately. Treated and control seed were planted in the greenhouse and in large-scale, non-replicated field trials at Guntersville and Marion Junction, AL, about 10 days after treatment.

RESULTS

Nonchemical control. Incidence of viable *Acremonium* decreased steadily from 90 to 0% in 1981 tall fescue seed stored 12 mo on the farm (Fig. 2). In the 1982 seed lot, viable endophyte levels decreased from 90 to 70 and 32% after 4 mo postharvest storage on the farm and at room temperature storage, respectively.

Incidence of viable endophyte in seed soaked in hot water at 50 C for 10 min combined with a 6-hr cool-water presoak at 5 C was significantly ($P = 0.05$) reduced (78.5 to 45%), without affecting seed germination. When the hot-water treatment time was increased to 20 min, endophyte levels were reduced significantly ($P = 0.05$), with 95% of the endophyte

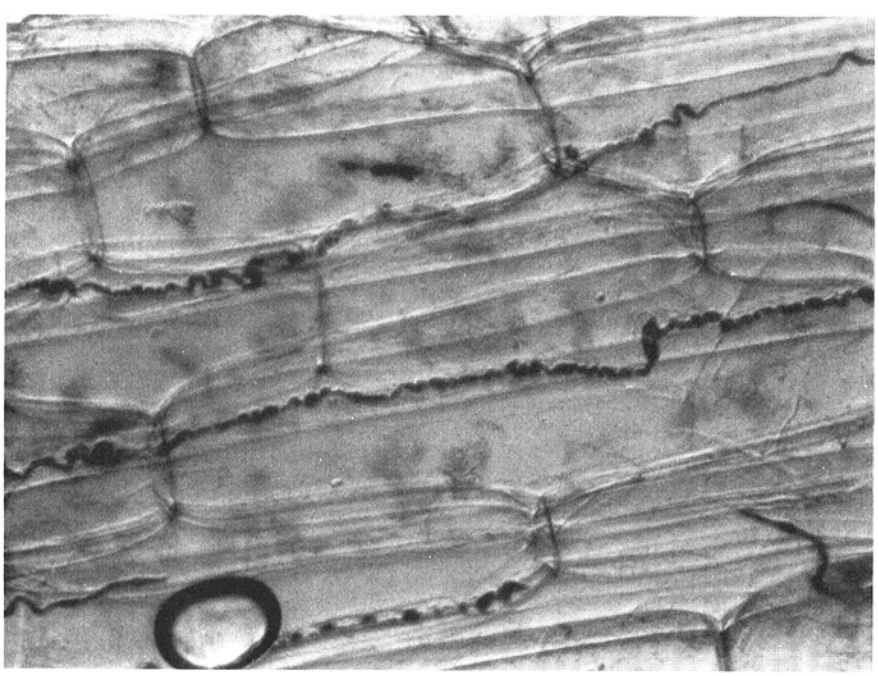

Fig. 1. Intercellular hyphae of the tall fescue endophyte in the mesophyll of tall fescue leaf sheath (×85).

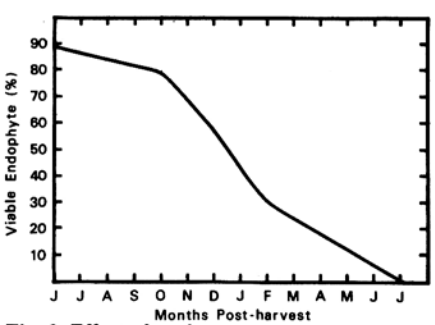

Fig. 2. Effect of on-farm storage on incidence of viable *Acremonium coenophialum* in tall fescue seed.

killed, but seed germination was significantly ($P = 0.05$) reduced (94.8 to 81.3%).

Chemical control. In preliminary screening tests, both commercial Black Belt seed lots had 90%+ seed infection; however, only 24% of the seedlings from the Black Belt seed were infected, whereas 79% of the seedlings from the commercial lot were infected. The difference from initial endophyte level apparently represented seed containing the nonviable endophyte. Both responded similarly to fungicide treatments (Table 1). Triadimenol and triadimefon were the most effective fungicides at the rates tested. Formulation differences did not affect endophyte control. When the rate of CGA-64251 was doubled, incidence of viable endophyte was not significantly affected (*unpublished*).

Endophyte levels in the 1982 commercial seed were similar to those the previous year. Triadimefon or triadimenol were equally effective ($P = 0.05$) in controlling *A. coenophialum* (Table 2). In contrast to results of the preliminary test (Tables 1 and 2), all fungicide treatments significantly lowered ($P = 0.01$) final germination. When the triadimefon study was repeated using the same seed lot aged 3 mo longer, germination and endophyte control were similar to those in the preliminary screening test (Table 2). Significant interactions ($P = 0.01$) were detected between age of the seed lot and fungicide performance evaluated either for endophyte control or phytotoxicity (final germination) (Table 2, Fig. 3).

Triadimenol or triadimefon applied with a commercial seed treater at 4.8 g a.i./kg seed resulted in 0% infection in both the greenhouse and north Alabama field planting (Table 3), but the field planting at Marion Junction had significantly higher levels of infection.

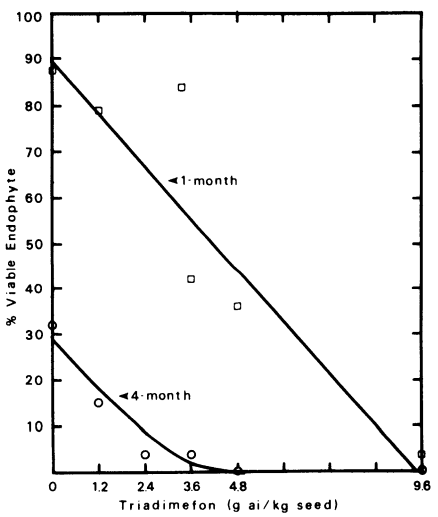

Fig. 3. Effect of triadimefon rate on viable tall fescue endophyte in seed treated 1 mo postharvest (□ = $y = 90.26 - 9.61X$, $r = 0.71$) and 4 mo postharvest (O = $y = 29.03 - 10.25X + 0.76X^2$, $r = 0.77$).

The commercial film-coater seed treater used in this study gave nonuniform coverage. Both treatments reduced seed germination by 30% in the germinator, but stand differences were not apparent in field plantings. Reductions in germination were usually associated with seed receiving heavy deposits of the fungicide (as evidenced by the presence of marker dye). Moderately and lightly treated seed were less severely affected by the seed-treatment formulation. All cases of viable endophyte surviving seed treatment in the greenhouse were associated with lightly treated seed.

DISCUSSION

Viable endophyte levels were effectively reduced by seed storage. This procedure is relatively inexpensive and is available to any individual who produces or purchases fescue seed. The recommended planting time for fescue in Alabama is within 3–5 mo of normal harvesttime; however, to ensure minimal infection levels in stored seed, on-farm storage should be extended at least to the next planting season (15–17 mo). Such factors as seed moisture content, temperature, and humidity of the storage environment may influence the rate of endophyte decline.

Table 1. Effects of systemic fungicides on viable *Acremonium coenophialum* levels and germination in commercially produced tall fescue seed

Treatment	Rate (g a.i./kg seed)	Infection[x] (%)	Germination rate[y] (%)
Control	···	78.5 ab[z]	100.0 a
Triadimenol	0.3	51.0 c	95.9 abc
Triadimefon	4.8	0.0 d	86.4 d
Etaconazole	0.1	79.0 a	97.6 abc
Metalaxyl	0.3	72.0 abc	97.2 abc
Carboxin + thiram	0.5 + 0.5	70.0 abc	96.8 ab

[x] Values are means of five replicates, five seedlings per replicate.
[y] Values are means of five replicates, 50 seeds per replicate.
[z] Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Effects of triadimenol and triadimefon on levels of viable *Acremonium coenophialum* and germination in tall fescue seed in the greenhouse

		1 mo Postharvest		4 mo Postharvest		
			Germination (%)[b]		Germination (%)	
Treatment	Rate (g a.i./kg seed)	Infected[a] (%)	Day 14	Infected (%)	Day 5	Day 14
Triadimenol	1.2	90.0	52.4	···	···	···
	2.4	57.8	52.4	···	···	···
	3.6	19.2	42.8	···	···	···
	4.8	15.0	41.8	···	···	···
Triadimefon	1.2	78.9	44.8	15	83.7	90.9
	2.4	83.8	37.2	4	70.8	84.7
	3.6	42.2	41.6	4	58.1	82.9
	4.8	35.8	31.6	0	63.8	85.6
	9.6	4.0	26.8	0	66.3	85.6
Control	···	87.5	69.2	32	82.8	90.6

[a] Values are means of five replicates, five seedlings per replicate.
[b] Values are means of five replicates, 50 seeds per replicate.

Table 3. Effects of triadimenol and triadimefon applied with a commercial seed treater on viable *Acremonium coenophialum* in tall fescue seed in Alabama

			Seed with viable endophyte (%)		
Treatment	Rate (g a.i./kg seed)	Coverage	Greenhouse[w]	Guntersville[x]	Marion Junction
Triadimenol	4.8	L[y]	5		
		M	0 } 1.7 a[z]	0	51.5
		D	0		
Triadimefon	4.8	L	5		
		M	0 } 1.7 a	3	47.5
		D	0		
Control (dye only)	···	L	81		
		M	68 } 71.0 b	62	66.5
		D	64		

[w] Values means of five replications, five seedlings per replicate.
[x] Values of 35 seedlings per location.
[y] L = light, M = medium, and D = dark.
[z] Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

The storage environment may be altered to shorten on-farm storage time and might, within a period of 8–10 mo, provide endophyte-free seed for areas of the Southeast where spring planting is a recommended practice. Seed germination, however, is adversely affected by prolonged storage. Increased planting rates to compensate for reduced germination and costs incurred during storage will be additional expenses in pasture establishment when using aged seed.

Hot-water treatments killed the endophyte in infected seed but seed germination was also affected. Similar results were reported by Latch and Christensen (8,9) on the endophyte of perennial ryegrass (*Lolium perenne* L.), which has been identified as *Acremonium* spp. (8). Germination rates would need to be determined for each seed lot receiving hot-water treatment so compensatory planting rates could be used. Energy and equipment requirements to dry hot-water-treated fescue would significantly affect the cost of seed treatment. It is questionable whether hot-water-treated seed would be competitive with fungicide-treated seed should these fungicides become available.

Systemic fungicides are required to disinfect seed because *Acremonium* mycelium is associated with the aleurone layer of the seed (12; E. M. Clark, *unpublished*). Sterol-inhibiting fungicides have been effective in reducing viable endophyte levels in seed, in potted plants, and in the field (16). Similar results have also been reported for control of the endophyte of perennial ryegrass seed (5,9). All formulations of triadimenol and triadimefon reduced viable endophyte levels in seed. In the United States, triadimefon is labeled for foliar disease control in fescue grown for seed, which should facilitate labeling for seed treatment; however, triadimenol is the preferred seed treatment. At current fungicide prices and assuming a triadimenol rate of 4.8 g a.i./kg of fescue seed, it would cost about an additional $13–18/ha to establish a fescue pasture with treated seed.

Triadimefon has been reported to interfere with gibberellin synthesis (15). Distorted seedlings were observed frequently with triadimefon rates of 4.8 g a.i./kg seed or greater; however, seedlings appeared to outgrow these symptoms a few weeks after emergence. Whether a chemical significantly reduced seed germination was partially dependent on seed sensitivity and rate (active ingredients) (Table 2). Newly harvested seed had the highest viable endophyte levels and required the greatest amount of fungicide to disinfect the seed. Short-term storage after harvest appears beneficial due to lowering of endophyte vigor, correspondingly reducing the fungicide rate necessary to disinfect and reducing the complicating factor of postharvest dormancy (Table 2). Formulation changes may overcome many of the phytotoxic effects. Furthermore, reduced seed germination caused by phytotoxicity can be offset by increasing the seeding rate.

Subsamples of seed from the same treatment lots (Table 3) differed significantly in levels of viable endophyte, depending on the planting location. This indicates that the fungicide does not kill the endophyte before planting but needs to be taken up by the imbibing seed or the emerging root system. Such factors as pH, cation-exchange capacity, organic matter, soil moisture, and microbial activity during germination could influence the amount of active ingredient available for plant uptake. These factors may have been responsible for the lack of observable stand differences in the field, whereas germination was significantly affected in the germinator. The commercial film coater did not give uniform seed coverage and this probably complicated the effect of soil factors. The effect of soil type on fungicide performance should be determined before commercial treatment recommendations are made.

There is no evidence for airborne inoculum because uninfested pastures adjacent to infested pastures have remained disease-free as long as 8 yr. The only known means of spread is by infected seed, but other means such as root grafting or mechanical transmission cannot be discounted. Minimum acceptable levels of infestation in a pasture for optimum animal performance is not known. To ensure long-term low levels of endophyte, seed used for planting new pastures should be free of viable *A. coenophialum*. Further research is needed to determine minimum effective fungicide rates, effects of soil characteristics on fungicide performance, and effects of storage environment on endophyte viability. The potential for greatly increasing animal production on tall fescue and the probability of future expansion of tall fescue hectarage if the endophyte is controlled make further research extremely important for all areas where tall fescue is adapted.

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