A Fungal Endophyte of Tall Fescue: Evaluation of Control Methods

M. R. Siegel, D. R. Varney, M. C. Johnson, W. C. Nesmith, R. C. Buckner, L. P. Bush, P. B. Burrus, II, and J. R. Hardison

First through fourth authors: professor, research associate, research plant pathologist, USDA-ARS, and associate extension professor, Department of Plant Pathology, University of Kentucky, Lexington 40546, respectively; fifth through seventh authors: research agronomist, USDA-ARS, professor, and research agronomist, USDA-ARS, Agronomy Department, University of Kentucky, respectively; eighth author: research plant pathologist, USDA-ARS, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

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ABSTRACT

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Treatment of tall fescue (*Festuca arundinacea*) field plots with either one late spring foliar application of benomyl, triadimefon, thiabendazole, etaconazol, or imazalil or one spring granular application of triadimefon or etaconazol did not result in control of the tall fescue fungal endophyte *Epichloë typhina* (also referred to as *Acremonium coenophialum*). Soil treatment of infected potted plants with bitertanol was partially successful. Storage at 21 C, short-term heat treatments, and fungicide treatments of infected seed were effective in destroying endophyte viability in greenhouse and field tests. Endophyte viability in infected seed was lost after 7–11 mo of storage at 21 C, but not after 19 mo of storage at temperatures <6 C. The heat treatment at 57 C for 40 min or at 49 C for 7 days controlled the endophyte, but 9–16% loss of seed viability occurred. Some seed treatment fungicides reduced germination and seedling vigor in the greenhouse. Certain formulated triazole fungicides were nontoxic or only slightly phytotoxic. These included a 40% dust formulation of triadimenol and a wettable powder formulation of bitertanol. Two flowable formulations of triadimenol were effective seed treatment fungicides that controlled the endophyte in a field test.

Additional key words: forage crops, triazole fungicides.

In a previous report (17) the tall fescue endophytic fungus *Epichloë typhina* (Pers.) Tul. (also referred to as *Acremonium coenophialum* Morgan-Jones & Gams), cause of summer toxicosis in cattle, is shown to be widely distributed in Kentucky and presumably in other regions where tall fescue is grown. The endophyte appears to have no effect on growth and reproduction of the host plant (17) and is transmitted only by seed (14,17). Furthermore, incidence of the endophyte in selected tall fescue plots did not change during the 4 yr of the study. Thus, the epidemiology of this fungus suggests strongly that any control methods that effectively reduce field infestations or endophyte viability in infected seed would result in a stable, low infestation level in existing or newly planted fields.

Recently, Johnson et al (8) reported that enzyme-linked immunosorbent assay (ELISA) could be used in a two-stage sampling procedure for identifying tall fescue seed lots that contain low percentages of endophyte-infected seed. Lots containing small amounts of infected seed (<5%) could be used to establish new pastures with low percentages of infected plants.

Viability of the fungus in tall fescue and perennial ryegrass seed decreases with storage (8,10,14,20). Although hot water treatment of seed rapidly destroys the fungus, there is a commensurate decrease on germination (10,20).

Chemical treatment of tall fescue and perennial ryegrass seed has been used successfully to eradicate the fungus in emerging seedlings

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(7,10,20). However, reduced germination occurred at effective rates (10,20). Certain sterol-inhibiting fungicides (16) have reduced endophyte infestation in potted plants and in the field (1,21).

In this study, chemical and nonchemical methods of control were evaluated for effectiveness in reducing or eradicating the endophyte in infected field-grown tall fescue plants and seed.

MATERIALS AND METHODS

The soil type used in greenhouse and field studies was a Maury silt loam. Two cultivars of tall fescue were used. G1-307 is an experimental strain derived from perennial (*Lolium perenne* L.) and annual (*L. multiflorum* Lam.) ryegrass × tall fescue hybrids. G1-316 is an experimental synthetic strain developed by increasing seven cultivar Kenhy tall fescue parental clones selected for low perloline alkaloid content.

Chemical control. Chemicals used in this study (Table 1) were first tested for antifungal activity in agar plate culture by the method of deWaard and Nistelrooy (19). The assay organism was a single-ascospore isolate of *Epichloë typhina* from bentgrass. Chemicals (technical grade) were added in organic solvents (methanol or acetone, 1% v/v) to Czapek's agar (at 50 C) supplemented with 0.25 g of yeast extract per liter. Agar plugs (5 mm in diameter) from the periphery of fungal cultures were placed in the center of the three assay plates for each of six concentrations tested. After 3 wk, radial growth was measured and ED_{50} and minimum inhibitory concentration (MIC) in micrograms per milliliter were determined from logarithmic probability plots.

Eradication of the endophyte in plants was studied by removing individual mature infected plants from the field and growing them in the greenhouse in pots containing steamed soil mixed with benomyl or bitertanol (2.5 and 5.0 mg/g dry soil, respectively). The plants were cut back to 5 mm 7–10 days after treatment and the stems were analyzed for the endophyte (zero time). At various times up to 55 wk, the regrowth was sampled and stem tissues were tested. All endophyte analyses, unless otherwise stated, were conducted by using the ELISA technique as previously described (9).

In a field study, an 8-yr-old sward of endophyte-infected (>98%) G1-307 tall fescue (managed for hay-pasturage [HP]) was treated with one foliar application in mid-June (2 wk after first cutting) of triadimefon (50W), thiabendazole (43F), or benomyl (50W), at 42 g a.i./93 m^2 and imazalil (5.8EC) or ectaconazol (10W) at 4 g a.i./93 m^2. Fungicides were applied with a 1.9-m boom (four nozzles) attached to a backpack sprayer pressurized with CO_2 propellant. Treatments were arranged in a randomized complete block design with three blocks and 2.5 × 7.7-m plots. After 8 wk new stem tissue was tested for the endophyte and pyrrolizidine (*N*-formyl and *N*-acetyl loline) alkaloid content (5). The accumulation of these alkaloids is associated with endophyte infection (5).

Fungicide treatment plots were established in a 9-yr-old sward of infected G1-307 tall fescue. Fungicide treatments were made on 1 April or 27 May 1983 and consisted of one application of granular triadimefon (0.5%) or propiconazol (2.5%) at the rates of 0, 2.6, 4.5, 6.7 kg a.i./ha in a randomized split block design with three blocks and 2.8 × 4.6-m plots. The April and May application plots were

TABLE 1. Effect of fungicides on the radial growth of *Epichloë typhina* in agar culture[a]

Class	Compound	Common name	Mfr.	Rate (μg/ml) ED_{50}	Rate (μg/ml) MIC[b]
Triazole	Triadimefon	Bayleton	Mobay	10.2	50
	Triadimenol	Baytan	Mobay	3.4	50
	Bitertanol	Baycor	Mobay	0.38	5
	Etaconazol	Vangard	Ciba Geigy	0.05	0.4
	Propiconazol	Tilt	Ciba Geigy	0.02	0.2
Benzimidazole	Benomyl	Benlate	DuPont	1.3	5
	Thiabendzole	Mertect	Merck	5.0	>50
	Thiophanate-methyl	Topsin-M	Pennwalt	16.5	>50
Pyrimidine	Fenarimol	Rubigan	Lilly	0.3	5
	Nuarimol	Trimidal	Lilly	0.5	3
Imidazole	Imazalil	Fungaflor	Janssen	0.015	0.06
	Prochloraz	Sportak	Boots Hercules	0.01	0.1
Piperazine	Triforine	Funginex	Chevron	28	>100
Carboxanilide	Carboxin	Vitavax	Uniroyal	25	>80

[a] Bentgrass isolate of *E. typhina* grown on Czapek's agar + 0.25 g yeast extract in the presence or absence of technical fungicide.
[b] MIC = minimum inhibitory concentration (100% inhibition of growth).

TABLE 2. Control of the tall fescue fungal endophyte by treatments with fungicides[a]

Compound and formulation	Tests (no.)	Highest effective conc. tested (g a.i/kg seed)[b]	Reduction of infection (%)[c]
Triadimenol (40D)	1	5.2	100
Triadimenol (15F)	3	5.2	100,96,100
Triadimenol (40F)	2	5.2	100,100
Bitertanol (25W)	2	5.2	100,97
Triadimefon (50W)	1	5.2	100
Prochloraz (40EC)	1	3.9	74
Propiconazol (10W)	1	1.95	96
Propiconazol (10EC)	1	2.6	100
Etaconazol (10W)	1	1.95	77
Nuarimol (5EC)	1	2.6	21
Imazalil (5.8EC)	1	1.95	23

[a] Analysis of dried stem tissue from each of thirty 10- to 12-wk-old seedlings grown in the greenhouse (five plants/10.2-cm-diameter pot).
[b] Fungicides applied in water using 0.30–1.0 ml per gram of seed.
[c] Seeds (Gl-307 or 316) were >98% infected. The untreated control seedlings had 83–100% infection.

TABLE 3. Effect of triazole fungicide seed treatments on seed germination, seedling growth, and control of the tall fescue fungal endophyte[u]

Compound	Fungicide rate (g a.i./kg seed)	Germ.[v] (%)	Height[w] (mm)	Control[x] (%)
Test 1	None	78 bcd	66 a[y]	
Triadimenol[z]				
15F	2.6	65 cde	39 cd	85
	3.9	66 cde	35 de	100
	5.2	49 f	22 f	100
30F	2.6	71 cde	58 b	34
	3.9	82 abc	42 cd	94
	5.2	77 bcd	39 cd	100
Triadimefon				
50W	3.9	75 bcd	37 cde	69
	5.2	76 bcd	32 e	96
Test 2	None	82 a	65 a	
Bitertanol				
25W	2.6	83 a	61 a	82
	3.9	77 a	63 a	90
	5.2	85 a	63 a	100
Test 3	None	88 a	67 a	
Triadimenol				
30D	3.9	84 a	51 b	88
40D	5.2	82 a	47 b	100
Propiconazol				
10W	1.3	70 b	26 e	79
	1.95	54 c	25 c	100

[u] Seed treated with fungicide-water (1 mg/g seed) and air-dried or with dusts and then seeded in pots (greenhouse). Stems from thirty 10- to 12-wk-old seedlings were tested for the endophyte.
[v] Percent germination determined in pots containing sand (50 seeds per pot).
[w] Height (mm) determined 13 days after seeding in pots containing sand (12 hr light/dark, 21 ± 1 C).
[x] Percent infection in control: test 1 = 86%, test 2 = 86%, and test 3 = 83%.
[y] For each test mean values within a column followed by the same letter are not significantly different (by Duncan's new multiple range test performed on log-transformed data, $P = 0.05$).
[z] Formulated triadimenol (Baytan) supplied by Gustafson, Inc., Dallas, TX.

managed for seed production (SP) and HP, respectively. Seed panicles were collected from the April-treated plots and tested for endophyte 10 wk post-treatment (15 June). These plots were then mowed. The May-treated plots were mowed 2 wk prior to granular application. Both application plots were mowed on 18 July and 15 September. Regenerated vegetative stems were collected from all plots on 10 November 1983 and tested for the endophyte.

Fungicides (see Tables 2 and 3 for names and rates) were applied to G1-316 seed that was >98% infected. Formulated fungicides were suspended in water at various ratios of water to seed (0.3–1.0 ml H_2O/g of seed), except for triadimenol which was also applied as a dust. After mixing the seed and fungicides for 4 min the wetted seed were air-dried by suction on a screen and then by an air stream in a hood. After being air-dried for 24 hr, the seed was planted in 10.2-cm-diameter pots of steamed soil and plants were thinned to six plants per pot (30 plants per treatment). After 8–12 wk, stems were tested for the endophyte. In some experiments, percent germination and height after 14 days of growth in sand or on blotter paper were determined.

Nontreated seed and seed treated with certain formulations of triadimenol were field-planted in the fall and spring in three replications of 3-m rows each. At the appropriate times, stems from five plants per replicate were tested for the endophyte.

Nonchemical control (heat treatment). Control of the endophyte was studied by treating G1-307 seed (>98% infected) with hot moist air. The heat treatment method used was essentially the same as that described by Miller and McWhorter (11). Hot, moist air was supplied by intermittently injecting mixing steam into a recirculating air stream to produce the temperature desired while maintaining moisture-saturated air in a closed chamber. The seeds (~250 g) were held in a thin layer on a screen tray. Seed was treated at 43 C for 15 min followed by 10, 20, 40, or 60 min at 60 C with recirculating air at ~100% relative humidity (RH). Immediately after treatment, the seed was cooled and dried in a stream of dry air at 38 C for 1 hr. The treated seed was planted in the fall of 1981 in the field (lath house), and in January 1982 the seedlings were transplanted into pots in the greenhouse. After 4 wk, endophyte presence was determined for 40 stems per treatment.

Heat treatment was used to kill the endophyte in 30 kg of breeder's seed of cultivar Johnstone (>95% infected). Seed (stored 9 mo at ~20 C) was placed for 7 days in a chamber at 49 C, 45% RH (seed moisture maintained at 8%). Treated and nontreated seed were tested for germination and percent viable endophyte. Viable endophyte was determined in 6-wk-old seedlings.

Newly harvested seed of G1-307 with >98% infection was stored at various temperatures to study the effect of storage temperature on viability of the endophyte. Every 4 mo, for up to 19 mo, a portion of the seed was removed from storage and planted in pots to determine the percent germination and presence of the endophyte. Seed was also stored for 15 mo at −80, −20, 6 and 21 C and then planted in the field (lath house) in the fall. The following spring, stems of flowering plants were tested for the endophyte.

RESULTS

Control (chemical treatment). A number of the sterol-inhibiting fungicides (16) and benomyl had ED_{50} values of <5 ppm (Table 1). Etaconazol, propiconazol, imazalil, and prochloraz had MIC values of <1.0 ppm. Thiophanate methyl, triforine, and carboxin were not sufficiently fungitoxic to warrant their use as treatment compounds.

Only partial success was achieved in eradicating the endophyte in infected plants potted in soil containing bitertanol (Table 4). In test 1, bitertanol was effective 14–28 wk after treatment. In test 2, transient (fungistatic) control was found in 10 plants after 14 wk, but the fungus reappeared in ~50% of the plants 28 wk after treatment. Benomyl gave no control in this experiment, but in other tests between 1980 and 1982 (*unpublished*) 10–25% of the plants treated remained free of the endophyte 1 yr post-treatment. No phytotoxicity occurred in these tests.

In the 8-yr-old stand of infected tall fescue, one foliar application with triadimefon, thiabendazole, benomyl, imazalil, or etaconazol was ineffective in reducing the levels of endophyte or pyrrolizidine alkaloids in new stem growth 8 wk post-treatment.

When granular applications of triadimefon or propiconazol were applied to a 9-yr-old sward in April or May at 2.3, 4.5, or 6.7 kg a.i./ha there was no appreciable change in the number of infected seed panicles or vegetative stems collected in June or November. The amount of rainfall from 1 April to 15 June and from 16 June to 15 September 1983 was 47 and 13 cm, respectively. The mean daily high temperature from 15 June to 16 September was 32 C with 43 days having daily high temperatures of ≥32 C.

Treatment of seed with some fungicides successfully reduced the occurrence of the fungus in 10- to 12-wk-old seedlings (Table 2). The most effective seed treatment fungicides (95–100% control) belonged to the triazole class of chemicals, and included triadimefon, triadimenol, bitertanol, and propiconazol. The most effective fungicides, as determined in the agar plate assay (Table 1), were not necessarily the best seed treatment chemicals. For example, the effective fungicides triadimefon, triadimenol, and bitertanol had ED_{50} values of 10.2, 3.4, and 0.38 ppm, respectively, while fenarimol, nuarimol, imazalil, and prochloraz which had ED_{50} values of 0.01–0.5 ppm were ineffective. In addition, at the highest concentrations tested, most chemicals were phytotoxic as indicated by reduced germination and seedling height (*unpublished*).

While triazole fungicides were effective as seed treatments, the amount of phytotoxicity was dependent on the chemical as well as the formulation used (Table 3). At effective control rates (95–100%) all the fungicides except bitertanol reduced germination and/or growth of 13-day-old seedlings. While the dust formulation of triadimenol also reduced growth, it was the least phytotoxic next to bitertanol. After 10–12 wk of growth, smaller plants (~25% smaller) were observed at the 1.95 and 5.2 g a.i./kg seed rates of propiconazol and triadimenol (15F formulation), respectively. At effective control concentrations propiconazol and the 15F formulation of triadimenol also reduced seed germination.

The 15F (5.2 and 3.9 g a.i./kg seed) and 30F (5.2 a.i./kg seed) formulations of triadimenol also effectively eradicated the endophyte under field conditions. In one test, seeds treated with triadimefon (15F and 30F formulations) was planted in the fall and the plants were tested for the endophyte the next spring. In a second test, 15F-treated seed was planted in the summer and 10 wk later the plants were analyzed for the endophyte. In both tests, the 15F formulation reduced germination and hence seedling stand. The 30F formulation produced no noticeable phytotoxicity.

Nonchemical control (heat treatment). The data presented in Table 5 indicates that storage temperature affects viability of the endophyte in seed. After 19 mo of storage at −20 C and 6 C, 90–95% of the seedlings were infected. When seeds were stored at 21 C, the endophyte became nonviable within 7–11 mo, while at 10 C storage only 55% of the seedlings were infected after 19 mo of storage.

TABLE 4. Effect of bitertanol and benomyl on post-treatment control of the fungal endophyte in stems of potted tall fescue plants[a]

Compound	Plants infected/number tested at: 0 wk	14 wk	28 wk	35 wk	55 wk
Test 1					
No fungicide	4/4	4/4	4/4	4/4	4/4
Benomyl[b]	4/4	4/4	4/4	ND[d]	ND
Bitertanol[c]	4/4	2/4	0/4	0/4	0/4
Test 2					
No fungicide	9/10	9/10	9/10	9/10	
Bitertanol[c]	10/10	0/10	5/10	3/10	
Bitertanol[e]	10/10	1/10	7/10	5/9[f]	

[a] Field-grown G1-307 tall fescue transplanted to fungicide-treated soil 1 November 1981 or November 1982 (test 2).
[b] 2.5 mg of fungicide/gm soil, 50W.
[c] 2.5 mg of fungicide/gm soil, 25W.
[d] ND = not determined.
[e] 5.0 mg of fungicide/gm soil, 25W.
[f] One plant died.

Germination appeared to be little affected after 19 mo storage at any of the temperatures. After acceptance of this article for publication, storage data were obtained for 27 mo. Values for endophyte viability remained the same as those for 19 mo except for 10 C which decreased to 30%. In a second test, of 15 mo storage, high levels of viable endophyte were found only in the seed stored at −80, −20, and 10 C but not at 21 C.

Treatment of seed at higher temperatures for short periods of time resulted in reduced viability of the endophyte (Table 6). After 40 min of moist heat treatment at 57 C, the fungus was not recovered in seedlings grown from the treated seed. However, germination was reduced 16% after this treatment.

In the spring of 1983, it became imperative to destroy the viability of the endophyte in ~30 kg of cultivar Johnstone breeder's seed. This seed was scheduled to be planted in August 1983 to produce endophyte-free foundation seed in 1984. The Johnstone tall fescue seed is a blend of the low perloline lines G1-307 and G1-316 and was 95% infected. The seeds were treated for 7 days at 49 C. In a pretreatment test, 25% of 120 seedlings were infected. After treatment, the endophyte could not be detected in any of 540 seedlings examined. Germination prior to treatment was 80% and after treatment 71% (9% loss of viability).

DISCUSSION

Both chemical and nonchemical treatments of infected seed or tall fescue plants were shown to be partially or totally effective in control of the endophyte. Of the chemical treatment methods evaluated, seed treatment with certain triazole fungicides appeared to be the most feasible from the standpoint of cost, lack of significant phytotoxicity, and effectiveness of control.

The dust formulation of triadimenol (Baytan) and the W formulation of bitertanol (Baycor) best fit this feasibility category. The use of a dust formulation has an added advantage of not requiring redrying of seed and permitting grower application just prior to planting. The latter consideration may be important in minimizing phytotoxicity in stored treated seed. Williams et al (20) have also reported the effectiveness of triadimefon (50W) and triadimenol (30F) in destroying endophyte viability in seed. In addition, etaconazol, propiconazol, and prochloraz have been shown to control the perennial ryegrass endophyte when used as seed treatment fungicides (7,10).

Neither soil fungicide treatment of potted plants nor field applications of foliar sprays or granular fungicides effectively controlled the endophyte. The objective of the field treatments was to greatly reduce or eliminate infestations in established pastures; this in turn would reduce the symptoms of summer toxicosis. Backman et al (1) demonstrated that infestation levels and toxicosis symptoms in cattle were reduced when grazing paddocks were treated with granular triadimefon and propiconazol at 1.8 and 4.2 kg a.i./ha, respectively. However, the levels of infestation returned to those of the pretreatment 7.5 mo post-treatment. Our failure to demonstrate significantly reduced infection in flowering panicles or vegetative stems of granular-treated tall fescue plants may have been due to the untimely application, where the endophyte escaped in the actively growing culms, to excessive spring rainfall which leached away the fungicides, or to the hot dry summer. Regardless, field application of granular fungicides does not seem feasible, because the yearly costs to apply effective concentrations are prohibitively large.

The combined use of granular and foliar fungicides eliminated the endophyte in infected potted greenhouse plants (21). Either granular or foliar application alone was ineffective. In our study, partial success in eradicating the endophyte from potted plants was achieved with soil application of bitertanol. Because some fungistatic effects occurred it is important that soil fungicide tests with other chemicals be carried out for a minimum of 28 wk. Chemical treatment of potted plants may be used to eradicate the endophyte in valuable breeding material.

Two nonchemical methods of endophyte control in seed are available. Short-term (~40 min) heat treatment successfully destroyed endophyte viability. Storage of seed in moderate heat for 1 wk killed the endophyte in cultivar Johnstone breeder's seed, with only a small loss (9%) of seed viability. Heat treatment seems feasible for eliminating the endophyte in experimental lots of seed. The loss of seed viability, however, may preclude commercial application.

The second method does not involve a treatment, but rather the use of seed lots certified to be <5% infested (8). This control method is highly feasible and may be preferred for cultivars such as Johnstone and Kenhy that will be certified to be low in endophyte and have superior agronomic characteristics (3,4) and animal nutrition performance (R. Hemken and J. Boling, *personal communication*).

It is clear that methods are now available that will result in the reduction or elimination of summer toxicosis. However, the possibility exists that this is not a desirable goal. Enhanced insect resistance has been reported for endophyte-infected perennial ryegrass (2,6,12,15). Removal of the endophyte from perennial ryegrass resulted in severe pasture predation by the Argentine stem weevil in New Zealand (13,15). Tall fescue grown in the U.S. appears to be relatively tolerant to insects. It is unknown whether this tolerance is due to the presence of the endophyte or to other factors such as tall fescue's vigorous growth. Even if endophyte-free tall fescue is susceptible to insect attack it is unknown whether the resulting damage would be of the same magnitude as that which occurs in perennial ryegrass.

TABLE 5. Effect of storage temperature on viability of the tall fescue fungal endophyte in seeds[a]

Temperature (C)	Endophyte viability and seed germination (%) after indicated months in storage for: 3 mo	7 mo	11 mo	15 mo	19 mo
−20	100 (86)[b]	100 (71)	100 (94)	100 (79)	90 (80)
6	100 (88)	90 (85)	85 (91)	90 (80)	95 (90)
10	90 (80)	100 (88)	80 (93)	75 (89)	45 (95)
21	95 (91)	60 (91)	0 (86)	0 (79)	0 (84)

[a] After storage, seeds planted in pots and after 10–12 wk stems from 30 plants tested for the endophyte.
[b] Figures in parentheses, percent germination determined on 80 seed grown in pots containing sterile soil.

TABLE 6. Effect of moist heat treatment of infected seeds of tall fescue on survival of the fungal endophyte and germination of the seed

Treatment time (min)[a]	Endophyte presence in plants[b]	Germination[c] (%)
0	+	91
10	+	92
20	+	90
40	−	83
60	−	75

[a] Seed (Gl-307, >98% infected) preconditioned at 43 C for 15 min and then treated at 57 C.
[b] Treated seed planted in fall 1981 (lath house) and in January 1982 transplanted to pots (greenhouse) for 3–4 weeks growth. Presence of the endophyte determined in two samples, each consisting of 20 stem sections.
[c] Germination of 200 seed planted in pots containing steamed soil.

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