Serological Reactivities of Endophytic Fungi from Tall Fescue and Perennial Ryegrass and of *Epichloë typhina*

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ABSTRACT

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There were few or no significant differences among absorbances elicited by mycelial preparations of six isolates of a tall fescue endophyte, an isolate of an endophyte from perennial ryegrass, and isolates of *Epichloë typhina* from bentgrass and wedgegrass when tested against an antiserum prepared to one of the tall fescue isolates (Ky-1) in a direct enzyme-linked immunosorbent assay (ELISA). Mycelial preparations of the various isolates were tested at concentrations ranging from 1 μg/ml to 1 mg/ml. All tall fescue isolates elicited absorbances that were not significantly different from each other at any given mycelial dry weight concentration. Mycelial homogenates of the endophyte from perennial ryegrass and the two isolates of *E. typhina* appeared to be less reactive than the isolates of tall fescue endophyte at the lower mycelial dry weight concentrations tested. Our ELISA system easily detected the perennial ryegrass endophyte in situ.

Recently, some animal maladies have been associated with the presence of endophytic fungi in grass pastures (1,6). A seed-transmitted fungus in tall fescue (*Festuca arundinacea* Schreb.) is considered directly or indirectly responsible for fescue toxicity syndrome (2,15,18). A similar endophyte found in perennial ryegrass (*Lolium perenne* L.) pastures in New Zealand has been implicated in a neurological disorder of sheep known as staggers (5,10). If isolates of the endophyte from different regions are antigenically similar, the use of a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) would be possible for endophyte detection in a tall fescue seed certification program (7,8).

Endophyte-infected tall fescue and perennial ryegrass plants do not show external symptoms of infection. In contrast, grasses susceptible to a plant disease called "choke," caused by *Epichloë typhina* (Pers.) Tul., a member of the Clavicipitaceae, may exhibit external stromata of the ascigerous and conidial states on infected culms (3,19). Many reports suggest a close relationship or identity of these endophytes, which contribute to poor animal performance, with *E. typhina* (1,13,16). In 1977, Bacon et al (1) identified the tall fescue endophyte as *E. typhina*, even though the sexual stage of the fungus has never been reported on tall fescue. Morgan-Jones and Gams (11) considered the tall fescue endophyte to be closely related but not identical to *E. typhina* and named the endophyte *Acremonium coenophialum* Morgan-Jones & W. Gams. On the basis of the similarities between the endophytic fungi in *Festuca* and *Lolium* species (13,14,16,17) and the work of Morgan-Jones and Gams (11), *A. loliae* Latch, Christensen, & Samuels has been suggested as a name for the perennial ryegrass endophyte associated with sheep staggers (9).

The purpose of this work was to determine the relative serological reactivities, as measured by ELISA, of isolates of the tall fescue endophyte from different locations in the southeastern United States, an isolate of an endophyte from a New Zealand cultivar of perennial ryegrass, and isolates of *E. typhina* from wedgegrass and bentgrass.

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MATERIALS AND METHODS

Isolates. Six isolates of the tall fescue endophyte from Alabama, Georgia, Kentucky, and Tennessee; an isolate of an endophyte from perennial ryegrass cultivar Ellett from New Zealand; and strains of *E. typhina* cultured from single ascospores from prairie wedgegrass (*Sphenopholis obtusata* (Michx.) Scribn.) and bentgrass (*Agrostis perennans* L.) were obtained (Table 1). In addition, a culture of *Acremonium strictum* W. Gams isolated from sorghum (*Sorghum bicolor* L.) (12) was supplied by R. A. Frederiksen. All cultures were maintained on a cornmeal agar medium as described previously (8).

Mycelial homogenates. Mycelial homogenates of each isolate were obtained by the procedure outlined under antigen production as described by Johnson et al (8). Because of limited mycelial growth of the perennial ryegrass endophyte in the M-43 medium (1), an alternate liquid medium was used for this isolate. This medium consisted of 50 g of sorbitol, 30 g of dextrose, 5 g of glutamic acid, 5 g of proline, 2 g of yeast extract, 1 g of KH_2PO_4, 0.3 g of $MgSO_4 \cdot 7H_2O$, and 1,000 ml of distilled water. After 6 wk of growth, all cultures were harvested, filtered, and washed (8). Mycelial residues were homogenized in a Waring Blendor for 30–60 sec, subjected to two centrifugation steps (8), and freeze-dried.

ELISA. The ELISA procedure described previously (7) was followed with only slight modifications. The antiserum was prepared against the Ky-1 isolate (Table 1) in the same manner as described (8) but was produced in a different rabbit. Conjugate was prepared with alkaline phosphatase (Type VII-T, Sigma) as outlined by Voller et al (21).

Table 1. Designation, geographic origin, host grass, and source of fungal isolates used as antigens

Isolate	Origin	Source[a]
Ky-1	Lexington, KY	Tall fescue pith (RAC)
Tenn-1	Knoxville, TN	Tall fescue seed (HER)
Tenn-2	Knoxville, TN	Tall fescue callus culture (BVC)
Ala-1	Auburn, AL	Tall fescue leaf sheath (EMC)
Ala-2	Selma, AL	Tall fescue leaf sheath (EMC)
Ga-238	Athens, GA	Tall fescue pith (CWB)
Ellett	New Zealand	Perennial ryegrass leaf sheath (MCJ)
316	Athens, GA	Wedgegrass ascospore (CWB)
9091-2	Athens, GA	Bentgrass ascospore (CWB)

[a] RAC = R. A. Chapman, Department of Plant Pathology, University of Kentucky, Lexington; HER = H. E. Reed, Department of Entomology and Plant Pathology, University of Tennessee, Knoxville; BVC = B. V. Conger, Department of Plant and Soil Science, University of Tennessee, Knoxville; EMC = E. M. Clark, Department of Plant Pathology, Auburn University, Auburn, AL; CWB = C. W. Bacon, Field Crops Laboratory, R. Russell Agriculture Research Center, USDA, ARS, Athens, GA; and MCJ = M. C. Johnson, Department of Plant Pathology, University of Kentucky, Lexington.

Table 2. Enzyme-linked immunosorbent assay for mycelial dry weight preparations of tall fescue endophyte isolates[y]

Isolate	Absorbance at 405 nm as related to mycelial dry weight			
	1 μg/ml	10 μg/ml	100 μg/ml	1 mg/ml
Ky-1	0.109 a[z]	0.199 a	0.348 a	0.477 a
Tenn-1	0.064 a	0.138 a	0.367 a	0.577 a
Tenn-2	0.095 a	0.165 a	0.324 a	0.416 a
Ala-1	0.076 a	0.147 a	0.322 a	0.467 a
Ala-2	0.101 a	0.155 a	0.344 a	0.687 a
GA-238	0.105 a	0.172 a	0.435 a	0.636 a

[y] Antiserum prepared against Ky-1 isolate.
[z] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Relative serological reactivities of tall fescue endophyte isolates, an endophyte of perennial ryegrass (Ellett), and *Epichloë typhina* from wedgegrass (316) and bentgrass (9091-2)

Isolate	Absorbance at 405 nm as related to mycelial dry weight[w]			
	1 μg/ml	10 μg/ml	100 μg/ml	1 mg/ml
Tall fescue endophytes[x]	0.092 a[y]	0.163 a	0.357 a	0.543 a
Ellett	0.060 ab	0.085 b	0.222 b	0.569 a
316	0.039 b	0.096 b	0.239 b	0.421 a
9091-2	0.056 ab	0.090 b	0.278 ab	0.551 a
AST[z]	0.006	0.005	0.007	0.010

[w] Mean absorbance of phosphate-buffered saline controls was 0.012.
[x] Mean absorbance of all tall fescue isolates at each concentration.
[y] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z] *Acremonium strictum* isolated from sorghum.

Freeze-dried mycelial preparations of the various fungal isolates (Table 1) were ground by mortar and pestle and dilutions made in phosphate-buffered saline containing 0.05% Tween 20 and 2% polyvinyl pyrrolidone, mol wt 40,000 (PBS-Tween-PVP). Dilution series of each isolate were replicated four times among eight ELISA plates. A dilution series of the Ky-1 isolate was tested on all eight plates to standardize absorbance values. In addition, a dilution series of the *A. strictum* isolate (AST) was placed on the ELISA plates to serve as a negative control. Absorbances were measured about 20 min after the substrate solution was added.

An additional experiment was conducted to determine the effectiveness of our ELISA system in detecting the perennial ryegrass endophyte in situ. Seeds of endophyte-infected perennial ryegrass cultivar Ellett and endophyte-free cultivar Ruanui, as well as tall fescue seed infected with its respective endophyte, were assayed on an individual seed basis (7). The endophyte status of the perennial ryegrass cultivars had been determined by microscopic examination of leaf sheaths (6).

RESULTS AND DISCUSSION

Absorbances elicited in ELISA plates by the mycelial preparations of the six endophytes from tall fescue were not significantly different among isolates (Table 2). In addition, absorbances elicited by the perennial ryegrass endophyte (Ellett) and *E. typhina* isolates (316 and 9091-2) were not significantly different from the isolates of tall fescue endophyte at 1 mg/ml (Table 3). At 1, 10, and 100 μg/ml, however, the absorbances elicited by Ellett, 316, and 9091-2 were often significantly lower than those of the tall fescue endophyte isolates (Table 3). The lower absorbances associated with Ellett, 316, and 9091-2 at mycelial dry weight concentrations lower than 1 mg/ml suggest that the antigen(s) in these isolates may be qualitatively or quantitatively limiting for binding to the test serum compared with the homologous antigen (Ky-1) and the other tall fescue isolates. The mycelial preparations of *A. strictum*, isolated from sorghum, did not elicit absorbances higher than background levels (Table 3).

Individual endophyte-infected perennial ryegrass seeds elicited absorbance readings comparable to those elicited by individual endophyte-infected tall fescue seeds (Table 4).

The evidence that the endophytes from tall fescue and perennial ryegrass are both linked to animal disorders and are serologically similar is intriguing. The toxin(s) may be produced by the host plant as a phytoalexin-type response, or the endophytic fungi may produce toxins. This latter possibility is similar to the circumstances of an animal disorder associated with annual ryegrass (*L. rigidum* Gaudin) pastures (4,20) in which a bacterium (*Corynebacterium rathayi* (Smith) Dowson) in host plant tissues produces toxins.

Table 4. Enzyme-linked immunosorbent assay (ELISA) for individual seeds of endophyte-infected or endophyte-free tall fescue and perennial ryegrass[a]

Cultivar or type	ELISA result[b]	
	$A_{405\ nm}$	Range
Endophyte-infected		
Ellett perennial ryegrass	0.442	0.213–0.690[c]
307 Tall fescue	0.329	0.230–0.486[c]
Endophyte-free		
Ruanui perennial ryegrass	0.005	0.000–0.016[d]
Kenhy tall fescue	0.008	0.000–0.014[d]

[a] Samples consisted of single seeds extracted in 0.6 ml of PBS-Tween-PVP.
[b] All absorbance values were from the same ELISA plate. Samples were randomized on the plate.
[c] Range of values for 10 determinations.
[d] Range of values for five determinations.

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