Relationship Between Endophyte Infestation Level of Tall Fescue Seed Lots and *Rhizoctonia zeae* Seedling Disease

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ABSTRACT

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The objective of this research was to define the relationship between endophyte (*Acremonium coenophialum*) infestation level of tall fescue (*Festuca arundinacea*) seed lots and a seedling disease caused by *Rhizoctonia zeae*. Seeds were planted in soilless medium or medium amended with *R. zeae* cultures, and number of seedlings per three 5.0-cm cores was determined. In a preliminary experiment, percentage of loss (61%) was greater for Forager (a low-endophyte cultivar) than for a Kentucky 31 seed lot with an 85% endophyte infestation level (32%). In subsequent experiments, Kentucky 31 seed lots with either 0 or 85% endophyte infestation level were used to determine if high percentage of loss in Forager was due to the lack of endophyte infection or to cultivar differences. For two *R. zeae* isolates, the number of seedlings was significantly higher in controls than in *R. zeae* treatments. Interactions between endophyte level and treatment were significantly different for both isolates. Additional seed lots were used to determine the nature of the relationship between endophyte infestation level and seedling loss. The relationship between endophyte level and seedling number was linear for both *R. zeae* treatments and controls ($P = 0.0615$).

Tall fescue is a popular pasture grass in the midsouthern United States because of its ease of establishment, persistence under less than optimal conditions, and long grazing season (15). Fescue toxicosis of grazing animals is highly correlated with the presence of an endophytic fungus (*Acremonium coenophialum* Morgan-Jones and W. Gams). Since toxicosis results in significant losses in profits ($609 million per year in the United States) (15,24), many beef producers have renovated pastures and replanted with endophyte-free (E−) tall fescue (*Festuca arundinacea* Schreb.). However, pastures planted with E− seed are often difficult to establish and maintain (10). Seed lots of Kentucky-31 (K-31), the cultivar most commonly grown, typically have moderate to high endophyte infestation levels. Because of the correlation between pasture infestation level and animal toxicosis, a number of endophyte-free cultivars have been developed. However, stands of these endophyte-free cultivars are often difficult to establish and manage (10). In addition to reduced pest and pathogen resistance (5,12), endophyte-free pastures are more likely to be overgrazed by cattle (24).

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Endophyte-infected (E+) plants are more resistant to many abiotic and biotic stresses than are their E− counterparts. Endophyte-infected plants are better able to withstand drought (1,26) and recover more rapidly from drought stress than E− plants (1). Drought stress appears to increase production of pyrrolizidine and ergot alkaloids in E+ plants (3). Alkaloids may be responsible at least in part for animal toxicosis (2,24) and herbivore resistance (6,16). Many insect species that feed freely on E− foliage are unable to sustain populations on E+ fescue, as noted in recent reviews (5,12). Evidence for differential resistance to soil fauna is limited. Although they may appear unthrifty, pastures with a low incidence of E+ plants often have no visible disease or pest problems. Since general unthriftiness is often associated with soilborne problems, we have chosen to examine effects of endophyte infection on resistance to soilborne pathogens and pests.

The most striking differential resistance seen so far is to endoparasitic nematodes. Densities of many nematode species are significantly higher in E− pastures than in pastures with high endophyte infestation levels (21,26), and some nematode species appear to be unable to survive in E+ plant rhizospheres (8,17). The endoparasitic nematodes, *Meloidogyne marylandi* Jepson (17) and *M. graminis* (Sledge & Golden) Whitehead (9), both produce significantly higher populations on E− tall fescue plants than on E+ plants. Populations of *Pratylenchus scribneri* Steiner, a migratory-endoparasitic nematode, are severely reduced on E+ plants (17). Numbers of Japanese beetle larvae are also reduced on E+ plants (20). Numbers of Japanese beetle (*Popillia japonica* Newman) larvae were significantly higher in pots containing predominantly E− plants than in pots containing all or predominantly E+ plants (20).

Endophyte infection appears to influence resistance of tall fescue to some fungal pathogens. Incidence and severity of stem rust (caused by *Puccinia graminis* Pers.:Pers.) were independent of endophyte status of the plant (25); however, crown rust (caused by *Puccinia coronata* Corda) was more severe on E− than on E+ plants (9). Although differential resistance to *Rhizoctonia zeae* Voorhees due to endophyte infection level was not expressed in mature plants (11), stands of E− fescue planted in *Rhizoctonia*-infested soil are often sparse and uneven, suggesting a reduced resistance to seedling disease caused by *Rhizoctonia*. Effects of endophyte infection of seed on the seedling disease caused by this pathogen have not been determined. The objective of this research was to determine if endophyte infestation level of tall fescue seed lots influences the development of seedling disease caused by *R. zeae*. Preliminary findings have been reported (4,13).

MATERIALS AND METHODS

Seeds of tall fescue cultivars Forager and K-31 were obtained from commercial sources and stored at 4 C. Levels of viable endophyte were determined with both an in vitro assay (7,14) or protein A sandwich enzyme-linked immunosorbent assay (PAS-ELISA) (22) of 8- to 10-wk-old plants. To generate a K-31 seed lot with low endophyte infestation level (hereafter referred to as 0%), one lot was stored at room temperature (RT) (about 22 C) for 1 yr.

Cultures. Two isolates of *R. zeae* (RZ112J and FC1) were obtained from L. T. Lucas (North Carolina State University) and were maintained on potato-dextrose agar with periodic transfers. Cultures for greenhouse studies were grown in 250-ml Erlenmeyer flasks on Forager seed (18). Deionized, distilled water (20 ml) was added to 10 g of seed, and flasks were autoclaved at 121 C for 20 min. Three 5-mm disks from a 2-day-old *R. zeae* culture grown on potato-dextrose agar were added to each flask. Flasks were incubated in the dark at RT for 14 days. Contents of the flasks were mixed with soilless medium (Promix, Premier Brands, Inc., Stamford, CT) (about 600 g, air-dried weight) and placed into a greenhouse flat. Seeds (10 g per flat) were evenly distributed on the surface and covered with a 2.0-cm layer of Promix. After a 10-day incubation in the greenhouse (with an average temperature 30 C), three cores (5.0 cm in diameter) per flat were harvested, and the total number of seedlings per three cores was determined.

Experimental designs. In preliminary experiments Forager (a low-endophyte cultivar) and K-31 were used. Endophyte infestation level of the K-31 seed lot was 85%. Treatments were arranged in an incomplete block design, with 10 blocks for the *R. zeae* treatments and five for controls. The experiment was repeated three times for a total of 30 blocks (*R. zeae* treatment) and 15 blocks (control).

The consistency of preparation of *R. zeae*-amended soilless medium and the efficiency of sampling techniques were not known; therefore, 30 blocks of the *R. zeae* treatment were used in the preliminary experiment. At the conclusion of this experiment, an estimation of power of the test (23) was used to determine the minimum number of replications needed. Power of the test uses data from preliminary experiments to predict probability of detecting differences if differences of a certain magnitude exist. The estimation of power of the test (23) indicated that as few as three blocks per repetition could be used in these experiments. However, since a nonlinear relationship was possible, we chose to use at least four blocks in subsequent experiments. Also, in these experiments (hereafter referred to as RCB experiments), only K-31 seed lots were used, and blocks were arranged in a randomized complete block design; each experiment was repeated three times.

In RCB experiments with two seed lots (0 and 85%), two treatments (control and *R. zeae*) and five blocks were used. In an additional RCB experiment, three seed lots were used: the 0% endophyte used in the first RCB experiment, a commercial seed lot (50% viable endophyte), and a 1:1 mixture of these seed lots. Treatments were the same as in previous experiments; however, four blocks were used. For data analysis each repetition of the experiment was treated as a true replication in time; thus, $n = 12$ or $n = 15$.

Data analysis. The General Linear Mixed Models procedure (19) was used to analyze data from the preliminary experiment and also a combined data set (augmentation of previous designs [23]) from all RCB experiments with isolate RZ112J. Data from RCB experiments were analyzed with the General Linear Models procedure (SAS Institute, Cary, NC).

Table 1. Cultivar differences in number of fescue seedlings surviving in control or *Rhizoctonia zeae*-amended soilless medium

Cultivar	Endophyte[a] (%)	*N*	Treatment[b]	Seedlings[c] (no.)	Loss (%)
Forager	0	15	Control	234	...
Forager	0	30	*R. zeae*	87	61
K-31	85	15	Control	323	...
K-31	85	30	*R. zeae*	221	32
Contrasts					
Forager versus K-31	$P = 0.0001$				
Control versus *R. zeae*	$P = 0.0001$				
Interaction	$P = 0.0367$				

[a] Endophyte infection levels were previously determined by in vitro assay or by protein A sandwich-enzyme-linked immunosorbent assay following greenhouse growout (8–10 wk).
[b] Seeds (10 g) were planted in soilless medium containing either *R. zeae* culture (isolate RZ112J) or autoclaved seed.
[c] Mean number of seedlings from all replications. Three cores (5.0 cm) per flat were harvested and total number of seedlings determined.

Table 2. Effects of seed lot infestation level on fescue seedling losses caused by *Rhizoctonia zeae* (isolate RZ112J)

Endophyte[a] (%)	*N*	Treatment[b]	Seedlings[c] (no.)	Loss (%)
0	15	Control	310	...
0	15	*R. zeae*	136	56
85	15	Control	383	...
85	15	*R. zeae*	336	12
Contrasts				
0 versus 85	$P = 0.0001$			
Control versus *R. zeae*	$P = 0.0001$			
Interaction	$P = 0.0003$			

[a] Endophyte infection levels were previously determined by in vitro assay or by protein A sandwich-enzyme-linked immunosorbent assay following greenhouse growout (8–10 wk).
[b] Seeds (10 g) were planted in soilless medium containing either *R. zeae* culture (isolate RZ112J) or autoclaved seed.
[c] Mean number of seedlings from all replications. Three cores (5.0 cm) per flat were harvested and total number of seedlings determined.

Table 3. Effects of seed lot infestation level on fescue seedling losses caused by *Rhizoctonia zeae* (isolate FC1)

Endophyte[a] (%)	*N*	Treatment[b]	Seedlings[c] (no.)	Loss (%)
0	15	Control	99	...
0	15	*R. zeae*	40	60
85	15	Control	125	...
85	15	*R. zeae*	98	22
Contrasts				
0 versus 85	$P = 0.0001$			
Control versus *R. zeae*	$P = 0.0001$			
Interaction	$P = 0.0181$			

[a] Endophyte infection levels were previously determined by in vitro assay or by protein A sandwich-enzyme-linked immunosorbent assay following greenhouse growout (8–10 wk).
[b] Seeds (10 g) were planted in soilless medium containing either *R. zeae* culture (isolate RZ112J) or autoclaved seed.
[c] Mean number of seedlings from all replications. Three cores (5.0 cm) per flat were harvested and total number of seedlings determined.

RESULTS

In the preliminary experiment, percentage of loss of seedlings was greater for Forager than for K-31 (Table 1). Endophyte was not viable in the K-31 seed lot stored at RT, and germination was reduced by about 30% (*data not shown*). In RCB experiments with two endophyte levels, interactions between endophyte level and treatment were significantly different for isolate RZ112J (Table 2). Similar results were obtained with an additional isolate (Table 3). Percentage of loss was at least 2.5 times greater for the seed lot with 0% endophyte than for the seed lot with 85%.

Germination was not different ($P < 0.05$) among the three lots used in the additional RCB experiment. There was a significant linear relationship ($P = 0.0197$) between endophyte level and seedling number for both treatments (Table 4). Relationships between seedling number and endophyte level were also linear for both treatments for the combined data (Table 5, Fig. 1).

DISCUSSION

In all experiments in this study, seedling loss was greatest for seed lots with low endophyte infestation levels. This relationship was consistent between isolates of *R. zeae* (Tables 2 and 3). Also, similar results have been obtained with *Rhizoctonia solani* Kühn (4). Since seed lots used in experiments with different isolates varied so greatly in endophyte infestation level, we tested seed lots with intermediate infestation levels. The relationship between endophyte infestation level and seedling number in this experiment was linear for both treatments and control. Treatment and control were also significantly different (Table 4). Similar results were obtained when data from two experiments (Tables 2 and 4) were combined (Table 5, Fig. 1). The slope of the line defined by control data was less ($P = 0.0001$) than the slope for *R. zeae* data, indicating a greater effect of endophyte infestation on *R. zeae* treatments than on controls (Fig. 1). Based on equations generated from these lines, *R. zeae* would not cause significant loss in a seed lot with 100% endophyte.

Forager is a low endophyte cultivar commonly used by Tennessee cattle producers for pasture renovation. Percentage of seedling loss due to *R. zeae* in Forager was twofold greater than in a seed lot of K-31 with an 85% endophyte infestation level (Table 1). Because of possible genotypic differences between cultivars, differences in seedling loss could not be ascribed solely to the endophyte. In RCB experiments, K-31 seed lots were used exclusively, but because we chose to use three commercial seed lots for these studies, it is not impossible that results from these experiments may also be confounded by genetic differ-

Table 4. Effects of seed lot infestation level on fescue seedling losses caused by *Rhizoctonia zeae* (isolate RZ112J)

Endophyte[a] (%)	*N*	Treatment[b]	Seedlings[c] (no.)	Loss (%)
0	12	Control	235	. . .
0	12	*R. zeae*	77	67
25	12	Control	289	. . .
25	12	*R. zeae*	155	46
50	12	Control	280	. . .
50	12	*R. zeae*	211	25

Contrasts

Linear	$P = 0.0001$
Quadratic	$P = 0.1940$
Control versus *R. zeae*	$P = 0.0001$
Interaction linear	$P = 0.0197$
Interaction quadratic	$P = 0.5394$

[a]Endophyte infection levels were previously determined by in vitro assay or by protein A sandwich–enzyme-linked immunosorbent assay following greenhouse growout (8–10 wk).
[b]Seeds (10 g) were planted in soilless medium containing either *R. zeae* culture (isolate RZ112J) or autoclaved seed.
[c]Mean number of seedlings from all replications. Three cores (5.0 cm) per flat were harvested and total number of seedlings determined.

Table 5. Analysis of variance of combined data sets (isolate RZ112J) from experiments defining the relationship between endophyte infestation of tall fescue seed lots and *Rhizoctonia zeae* seedling disease

Source	df	Mean squares	*P > F*
Time	1	59,585	0.0001
Replication (time)	4	70,190	0.0001
Block (replication time)	21	9,928	0.0007
Endophyte	3	127,538	0.0001
Linear	1	374,678	0.0001
Quadratic	1	3,683	0.3229
R. zeae	1	318,529	0.0001
R. zeae * endophyte	3	29,043	0.0001
Linear * *R. zeae*	1	13,350	0.0615
Quadratic * *R. zeae*	1	1,463	0.5327
Error	100	3,732	

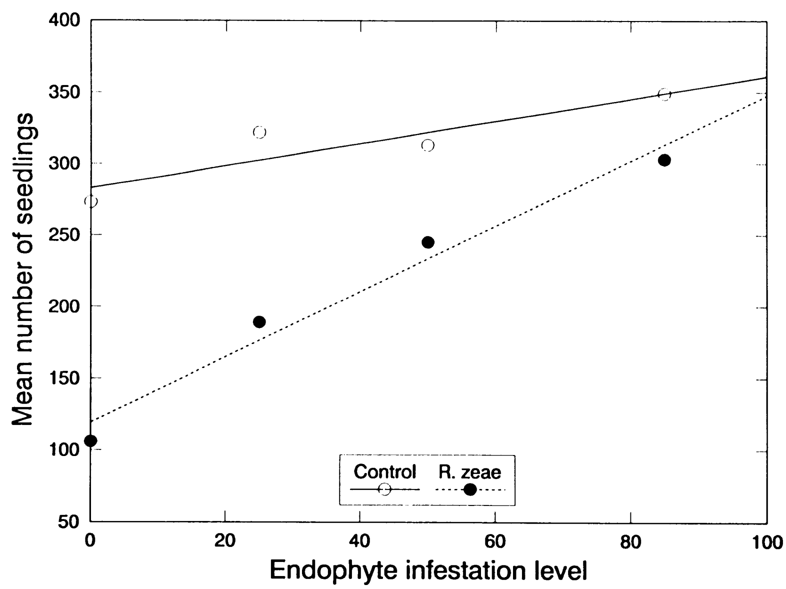

Fig. 1. Influence of endophyte infestation level on seedling number. Seeds were planted in a control or *Rhizoctonia zeae*-augmented soilless medium. Data from previous experiments (Tables 2 and 4) were combined and analyzed by General Linear Mixed Models (Table 5) (4). Estimated slope parameters between control and inoculated are significantly different ($P = 0.0001$).

ences. Commercial seed lots of K-31 are necessarily genetically diverse, since the plant is an obligate cross-pollinator, and because seed lots from many growers are bulked before sale. However, the linear interaction between seedling number and endophyte infestation level for the *R. zeae* treatment suggests that endophyte infestation level was the primary variable (Table 5, Fig. 1).

Given the results presented here, we believe that endophyte infestation level should be considered when planting fields in areas with known *Rhizoctonia* problems. Growers may opt to plant a seed lot with high endophyte and to reduce animal toxicosis problems by other strategies (e.g., overseeding with clover to dilute the amount of fescue in the diet). On the other hand, an increased seeding rate might compensate for seedling losses in a low-endophyte variety. Current research may lead to additional options in the future. For example, therapeutic agents may be found to counteract toxicosis in the animal. Also, many researchers are attempting to alter the endophyte so that toxicosis may be eliminated without loss of resistance to pests, pathogens, or drought.

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