Effect of Eradication of the Endophyte *Acremonium coenophialum* on Epidemics of Rhizoctonia Blight in Tall Fescue

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ABSTRACT

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The endophytic fungus *Acremonium coenophialum* was eradicated from selected germ plasms and genotypes of tall fescue by heat treatment or long-term storage (>3 yr) of seed or by fungicide treatment of tillers. This endophyte-free (EF) plant material was then compared with endophyte-infected (EI) plants to assess the progress of epidemics of Rhizoctonia blight in a greenhouse. No significant differences in area under disease progress curve (AUDPC) or AUDPC + area under crop recovery curve (AUCRC) values were detected between EF and EI plants. Two genotypes had significantly lower AUDPC + AUCRC values than other genotypes tested, but the status of endophyte infection had no significant effect on disease progress or crop recovery. In addition, no significant difference in response to *Rhizoctonia solani* was detected in plants of tall fescue cv. Arid grown from EF seed than from those grown from EI seed.

Tall fescue (*Festuca arundinacea* Schreb.) is a turf and forage grass grown on 12–14 million ha in the United States (6), where an estimated 90% of pastures are infested with the fungal endophyte *Acremonium coenophialum* Morgan-Jones & W. Gams (18). The endophyte forms a mutualistic, symbiotic relationship with tall fescue, resulting in increased growth, tolerance to drought, and deterrences to insect, nematode, and mammalian herbivory in specific populations of host plants (3,18).

Isolates of *A. coenophialum* and other endophytes of grasses have inhibited the growth of several fungal pathogens in vitro (17,20), but the observation of disease resistance in an endophyte-infected (EI) grass is limited to a single report (14). Recently, Gwinn and Gavin (12) observed that the survival of EI tall fescue seedlings was greater than that of endophyte-free (EF) seedlings in soil infested with *Rhizoctonia zeae* Voorhees. However, the mechanism of the enhanced survival (i.e., disease resistance or escape) was not elucidated. The paucity of reports on disease resistance in EI grasses has been explained in part by Siegel and Latch (17), who stated that "most endophyte isolates have a narrow spectrum of antifungal activity against grass pathogens and those that are most active are either from noncommercial grasses . . ., not widely disseminated . . . or may exist as biotypes having degrees of activity . . . ." This explanation is based on the assumption that antibiosis in vitro is an accurate measure of the potential for disease suppression in vivo. This assumption has not been tested with endophytes of grasses, but in general, only weak correlations between antibiosis in vitro and disease suppression in vivo have been observed with other micoorganisms (10). In addition to antibiosis, it is possible that endophytes may suppress disease through competition with pathogens for nutrients and/or space within the host plant or through the induction of host resistance. Since these mechanisms are difficult to detect in vitro, the monitoring of disease intensity on EI and EF genotypes and germ plasms of grasses is warranted.

Rhizoctonia blight or brown patch caused by *R. solani* Kühn is a severe disease of tall fescue grown in pastures and as a turfgrass in the southeastern United States (9). The pathogen causes leaf blight and crown rot, resulting in patches of necrotic turf that may exceed a meter in diameter. Management of Rhizoctonia blight is limited to fungicide applications and reductions in the duration of foliar wetness by decreasing shade and increasing air circulation around turfgrass swards (19). Although resistant cultivars of tall fescue are not available, the progress of epidemics of Rhizoctonia blight may differ significantly among populations (7). Given the beneficial effects of infection by fungal endophytes on host grasses (3), the objective of this study was to determine the influence of eradication of *A. coenophialum* on epidemics of Rhizoctonia blight in selected populations and genotypes of tall fescue.

MATERIALS AND METHODS

Source of plant material. EI and EF seeds of the tall fescue cv. Arid were obtained from Jacklin Seed Company, Post Falls, Idaho. The seeds had been selected individually from EI and EF plants in field plots. Seeds of two experimental germ plasms of EI tall fescue, Georgia 5 (GA-5) and Georgia-Jesup Improved (GA-JESIMP), were harvested from seed-increase plots near Athens, Georgia. The EF version of GA-5 was produced by long-term storage of the EI seed (>3 yr), which reduced the viability of the endophyte (3). To eradicate the endophyte from GA-JESIMP, seeds were suspended in a desiccator on a wire frame at 47 C for 7 days above a mixure of 75% glycerol and 25% water that produced a relative

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humidity of 45% (4). After the eradication procedure, samples of the EI and EF seed were planted in field isolation plots near Athens, and seed was increased one generation. This increased seed was used in the current study. Samples of 100 8-wk-old seedlings derived from this seed were stained and examined microscopically for endophyte infection according to the procedure of Bacon et al (2). The endophyte infestation level was 85% for GA-5 EI, 96% for GA-JESIMP EI, and 0% for the EF germ plasms.

The EI tall fescue genotypes included in this study were 80-51, 80-85, 80-88, 80-89, 87-122, and 87-124. An EF clone of each genotype was produced by treating rooted ramets with the fungicide propiconazole according to the methods of De Battista et al (11). The agronomic characteristics and origin of each genotype have been described by Bouton et al (5).

Disease assessment. Three experiments were conducted in a growth chamber and greenhouse to assess differences among EI and EF tall fescue in response to infection by *R. solani*. For each experiment, plants were grown in a greenhouse in 10.2-cm-diameter Styrofoam cups containing granular calcined clay, watered daily, and fertilized weekly with a solution containing 434, 99, and 373 ppm of N, P, and K, respectively. In the first and second experiments, EI and EF seed were sown at a rate equivalent to 244 kg/ha for Arid or the two germ plasms. In the third experiment, a rooted tiller was planted in a cup for each EI and EF clone of each of the six genotypes tested. All plants were grown for 8 wk prior to inoculation and cut weekly with scissors to a height of 7 cm. To confirm the presence or absence of endophyte in the plant material tested, a tiller was randomly selected and removed from each of three cups of each set of EI and EF plants, and the leaf sheaths were microscopically examined for hyphae of *A. coenophialum* (15).

For each experiment, inoculum was prepared by culturing isolate R42 of *R. solani* AG-1 from tall fescue for 3 wk at 23 C on autoclaved rye grain (8). The grain was then air-dried for 12 hr in a laminar-flow microbe transfer hood, ground in a Wiley mill, and sieved to a particle size of 1–3 mm in diameter. The turf was inoculated by placing 1.5 g of the infested grain within the foliar canopy in the center of a cup. Four cups of each set of EI or EF plants were treated with inoculum, and one cup of each set served as a noninoculated control. Immediately after inoculation, the turf foliage was misted with water to runoff, and the cups were placed in a completely randomized design in a Plexiglas mist chamber constructed within a plant growth chamber. The turf was incubated at 100% RH with 14 hr of light (photon flux density of 350 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 30 C and 10 hr of dark at 24 C.

At intervals of 1, 2, 4, and 8 days postinoculation, one cup of each set of EI or EF plants was removed from the mist chamber and placed on a greenhouse bench, where the turf foliage was allowed to dry. After 24 hr, disease severity (percent necrotic foliage per cup) was estimated by the Horsfall-Barratt rating scale (13). Drying resulted in greater visual contrast between necrotic and nonnecrotic foliage, which facilitated estimates of disease severity. A plot of the disease severity values over time yielded a disease progress curve for each set of EI and EF plants. Each treatment was repeated four times over a 12-wk period (i.e., treatments were replicated over time rather than space) to provide five replications for statistical analysis. Each experiment was repeated once.

In the first and third experiments, crop recovery (the postepidemic regrowth of symptomless foliage) was assessed for cups of turf that were incubated in the mist chamber for 8 days, then transferred to a greenhouse bench for 4 wk. At weekly intervals during this period, the turf was cut with scissors to a height of 7 cm. The turf was irrigated and fertilized by placing the cups in flats of water or, at weekly intervals, in the fertilizer solution. Water and fertilizer entered the cups through drainage holes in the bottoms. This form of irrigation limited further disease development by preventing foliar wetness. Crop recovery was assessed at intervals of 1, 2, and 4 wk after the cups were placed in the greenhouse by estimating the percent necrotic foliage with the Horsfall-Barratt rating scale (13). Necrosis declined in most plants as a result of the growth of symptomless foliage from meristems that survived the epidemics. A plot of percent necrosis over time yielded crop recovery curves for each treatment-replication combination. Area under disease progress curve (AUDPC) and area under crop recovery curve (AUCRC) were calculated with the formula $\Sigma[(y_i + y_{i+1})/2]\,[t_{i+1} - t_i]$, where $i = 1,2,3,\ldots,n-1$, y_i is the amount of disease (necrosis), and t_i is the time of the ith rating (16).

Data analysis. Statistical calculations were performed by Statistical Analysis Software (SAS Institute Inc., Cary, NC) procedures. Values of AUDPC and AUDPC + AUCRC were subjected to analysis of variance at $P \leq 0.05$, with replicates, genotypes, or germ plasms and infection status (EI or EF) as factors. Values of AUCRC were not analyzed alone because the origins of the recovery curves were not equivalent for each treatment. Means were statistically separated by Duncan's least significant difference test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Eradication of the strain(s) of *A. coenophialum* that infected the germ plasms and genotypes of tall fescue used in this study had no effect on the progress of epidemics of Rhizoctonia blight (Table 1). Similarly, there was no significant difference in susceptibility to *R. solani* in the EF population of the cv. Arid vs. the EI population. We speculated that the endophyte may enhance postepidemic recovery (regrowth) of necrotic turf because of observations of endophyte-enhanced recovery from drought (1). However, crop recovery, which was

Table 1. Analysis of variance of area under disease progress curve (AUDPC) and AUDPC + area under crop recovery curve (AUCRC) for epidemics of Rhizoctonia blight on endophyte-infected and noninfected plants of tall fescue cv. Arid and selected germ plasms and genotypes

		Experiment 1		Experiment 2		Experiment 3	
Parameter	Source of variation[v]	MS[w]	P[w]	MS	P	MS	P
AUDPC	Germ plasms, genotypes	...[x]	...	8.03	0.93	3,139	0.08
	Infection status[y]	10,650	0.074	910	0.92	0.16	0.98
	Error	96,152		918		293	
AUDPC + AUCRC	Germ plasms, genotypes	...	...	ND[z]	ND	15,239,342	0.02
	Infection status	8,182	0.77	ND	ND	19,224	0.89
	Error	80,797		ND		996,843	

[v] Germ plasms were Georgia 5 and Georgia-Jesup Improved and genotypes were 80-51, 80-85, 80-88, 80-89, 87-122, and 87-124.
[w] MS = mean square, P = probability of observing more extreme values by stochastic variation.
[x] Only endophyte-infected and endophyte-free plants of cv. Arid were assessed.
[y] Endophyte-infected vs. endophyte-free plants.
[z] ND = not determined.

Table 2. Area under disease progress curve (AUDPC) and AUDPC + area under crop recovery curve (AUCRC) for epidemics of Rhizoctonia blight on six genotypes of tall fescue[y]

Genotype	AUDPC	AUDPC + AUCRC
80-89	443.8 a[z]	2,026.5 a
80-85	426.6 a	1,866.7 a
80-51	478.1 a	1,747.8 a
80-88	435.9 a	1,344.6 ab
87-124	326.5 a	1,045.2 b
87-122	273.4 a	1,034.4 b

[y] Data were pooled for each endophyte-infected and endophyte-free version of each genotype.
[z] Within a column, values followed by the same letter are not significantly different at $\alpha = 0.05$ according to Duncan's least significant difference mean separation procedure.

estimated by measuring AUDPC + AUCRC, was not significantly influenced by the endophyte (Table 1). Significantly lower values of AUDPC + AUCRC were associated with the clones 87-124 and 87-122 (Table 2), but the status of endophyte infection in these clones had no effect on disease progress or crop recovery.

The antagonistic activity of the endophyte strains in this study was unknown, and it is possible that the results may have been influenced by the existence of strains that produced little or no antagonism to *R. solani* in the plant material used. Nevertheless, the selection of germ plasms and genotypes without regard to individual strains of endophytes indicates that resistance to *R. solani* is not a common or easily detectable endophyte-mediated survival mechanism in tall fescue.

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LITERATURE CITED

1. Arechavaleta, M., Bacon, C. W., Hoveland, C. S., and Radcliffe, D. E. 1989. Effect of the tall fescue endophyte on plant response to environmental stress. Agron. J. 81:83-90.
2. Bacon, C. W., Porter, J. K., Robbins, J. D., and Luttrell, E. S. 1977. *Epichloe typhina* from toxic tall fescue grasses. Appl. Environ. Microbiol. 34:576-581.
3. Bacon, C. W., and Siegel, M. R. 1988. The endophyte of tall fescue. J. Prod. Agric. 1:45-55.
4. Bouton, J. H., Gates, R. N., Balesky, D. P., and Owsley, M. Yield and persistence of tall fescue in the southeastern coastal plain after removal of its endophyte. Agron. J. In press.
5. Bouton, J. H., Smith, S. R., Jr., and De Battista, J. P. 1992. Field screening for rhizome number in tall fescue. Crop Sci. 32:686-689.
6. Buckner, R. C. 1985. The fescues. Pages 233-240 in: Forages: The Science of Grassland. M. E. Heath, R. F. Barnes, and D. S. Metcalfe, eds. Iowa State University Press, Ames.
7. Burpee, L. L. 1992. Assessment of resistance to *Rhizoctonia solani* in tall fescue based on disease progress and crop recovery. Plant Dis. 76:1065-1068.
8. Burpee, L. L., and Goulty, L. G. 1984. Suppression of brown patch disease of creeping bentgrass by isolates of nonpathogenic *Rhizoctonia* spp. Phytopathology 74:692-694.
9. Burpee, L., and Martin, B. 1992. Biology of *Rhizoctonia* species associated with turfgrasses. Plant Dis. 76:112-117.
10. Cook, R. J., and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN.
11. De Battista, J. P., Bouton, J. H., Bacon, C. W., and Siegel, M. R. 1990. Rhizome and herbage production of endophyte-removed tall fescue clones and populations. Agron. J. 82:651-654.
12. Gwinn, K. D., and Gavin, A. M. 1992. Relationship between endophyte infestation level of tall fescue seed lots and *Rhizoctonia zeae* seedling disease. Plant Dis. 76:911-914.
13. Horsfall, J. G., and Cowling, E. B. 1978. Pathometry: The measurement of plant disease. Pages 119-136 in: Plant Disease: An Advanced Treatise. Vol. 2, How Disease Develops in Populations. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York.
14. Koshino, H., Togiya, S., Yoshihara, T., Sakamura, S., Shimanuki, T., Sato, T., and Tajimi, A. 1987. Four fungitoxic C-18 hydroxy unsaturated fatty acids from stromata of *Epichloe typhina*. Tetrahedron Lett. 28:73-76.
15. Saha, D. C., Jackson, M. A., and Johnson-Cicalese, J. M. 1988. A rapid staining method for detection of endophytic fungi in turf and forage grasses. Phytopathology 78:237-239.
16. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.
17. Siegel, M. R., and Latch, G. C. M. 1991. Expression of antifungal activity in agar culture by isolates of grass endophytes. Mycologia 83:529-537.
18. 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.
19. Smith, J. D., Jackson, N., and Woolhouse, A. R. 1989. Fungal Diseases of Amenity Turfgrasses. E. & F. N. Spon Ltd., New York.
20. White, J. F., Jr., and Cole, G. T. 1986. Endophyte-host associations in forage grasses. IV. The endophyte of *Festuca versuta*. Mycologia 78:102-107.