

Response of Field-Grown Tall Fescue Infected by *Acremonium coenophialum* to *Puccinia graminis* subsp. *graminicola*

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ABSTRACT

Welty, R. E., Barker, R. E., and Azevedo, M. D. 1993. Response of field-grown tall fescue infected by *Acremonium coenophialum* to *Puccinia graminis* subsp. *graminicola*. Plant Dis. 77:574-575.

Plants of tall fescue cultivar Kentucky 31 infected (E+) or not infected (E-) by the endophyte *Acremonium coenophialum* were evaluated for reaction to *Puccinia graminis* subsp. *graminicola* for 2 yr in field plots. Stem rust was assessed on the modified Cobb scale on five dates in 1991 and three dates in 1992. On each date, stem rust severity in susceptible plants of Kentucky 31 was similar for E+ and E- plants. On the final assessment in both years, the number of plants without stem rust (0% infection) was similar for E+ and E- plants. Based on these results, the presence of *A. coenophialum* in plants of Kentucky 31 did not influence stem rust severity or the number of stem rust-resistant plants. Thus, the stem rust reaction is not influenced by the presence of this endophyte in tall fescue.

A fungal endophyte, *Acremonium coenophialum* Morgan-Jones & W. Gams (11), grows intercellularly in foliage of tall fescue (*Festuca arundinacea* Schreb.) and is responsible for a fescue toxicity syndrome in cattle (3). It also produces toxic alkaloids in culture (2). Beneficial aspects of endophyte infection in field-grown tall fescue include increased resistance to insect feeding (16), drought tolerance, and persistence (4,15,18). In field experiments (12), increasing seeding rates of an endophyte-free cultivar (AU-Triumph) above those of an endophyte-infected cultivar (Kentucky 31) was not necessary for successful establishment of pastures. Interactions between endophyte-infected grasses and plant pathogens have been reported to reduce disease severity (7,8,10,19) and nematode reproduction rates (9,13,18). Other reports (5,6) have shown that endophyte infection has no effect on other pathogens.

Endophyte infection did not influence the stem rust infection type in seedlings

Cooperative investigations of the USDA-ARS and the Departments of Botany and Plant Pathology and of Crop and Soil Science, Oregon State University. Technical paper 9995 of the Oregon Agricultural Experiment Station.

Accepted for publication 29 January 1993.

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of tall fescue grown under controlled conditions in the greenhouse and inoculated with *Puccinia graminis* Pers.:Pers. subsp. *graminicola* Z. Urban (17). This study evaluated the response to the stem rust pathogen in endophyte-infected (E+) and endophyte-free (E-) tall fescue plants in field plots where the disease naturally occurs.

MATERIALS AND METHODS

Seeds from E+ and E- Kentucky 31 plants were surface-sterilized in sulfuric acid and distilled water (1:1, v/v) for 20 min and in 5.25% NaOCl containing 1% Tween 20 for 20 min. The seeds were rinsed several times with sterile deionized water between and after treatments to remove acid and chlorine residues. The excess water was drained off, and the seeds were air-dried. Individual seeds (150 per seed source) were transferred to test tubes containing potato-dextrose agar and incubated in a growth chamber in light ($50 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$) for 8 hr at 25 C and in the dark for 16 hr at 15 C, on a 24-hr cycle. After 7 wk, seedlings were examined, and mycelium growing into the agar was verified to be *A. coenophialum*. One hundred E+ and 100 E- seedlings were transplanted into fine-grade vermiculite in cone-shaped plastic containers (3.8×21 cm), with a single seedling in each container. Cones containing E+ and E- seedlings were placed in racks in a completely randomized design. A rack holding 98 cones was placed in a mist chamber for 7 days until

the plants were well rooted. The racks were then moved to a greenhouse at 20 ± 5 C.

In early November 1990, E+ and E- Kentucky 31 plants growing in the greenhouse were clipped to remove excess foliage, fertilized with an N-P-K solution (Peters 20:20:20, N content of 473 ppm), and moved outside to the north side of a headhouse to harden off. On 26 November 1990, each plant was removed from its single plastic container, and the plants were transplanted into field plots near Corvallis, Oregon. In this study, 20 E+ and 20 E- plants were planted on 1-m centers in each of five replicate rows of 40 plants each. The location of E+ and E- plants within each row was completely randomized. The plants were maintained for 2 yr. Lime and fertilizer were applied, following soil tests, as recommended for maintaining optimum plant growth. Weeds were controlled mechanically.

Inoculum of *P. g. graminicola* came from natural sources. The plants were observed for stem rust at 5- to 7-day intervals beginning in April 1991. Because of the late-November transplanting, most of the plants did not vernalize and grew vegetatively through the summer. This provided a longer season for observing stem rust development in 1991. Following the last assessment for stem rust in September 1991, the plants were cut with a rotary mower, and the foliar clippings were left in the field. The following spring (1992), the plants were observed for stem rust occurrence at 5- to 7-day intervals.

When stem rust developed, severity in each plant was assessed on the modified Cobb scale (14). Because panicles of tall fescue develop continuously, the assessment for each plant was an average based on examination of the seed head and the two top leaves on five to 10 tillers per plant.

The experiment was analyzed as a randomized complete block with endophyte infection as treatments and observation dates as repeated measures over time. Data were collected on individual plants and pooled for each replication on each

Table 1. Stem rust incidence and severity in endophyte-free (E-) and endophyte-infected (E+) plants of Kentucky 31 tall fescue grown in the field

	Stem rust incidence ^a			Stem rust severity (%) ^c		
	E-	E+	P value ^b	E-	E+	P value ^d
1991						
1 Aug.	82	77	0.001	
5 Aug.	87	86	0.570	
9 Aug.	93	93	0.780	9	8	0.540
19 Aug.	93	94	0.670	20	18	0.450
23 Aug.	94	94	0.780	26	28	0.500
29 Aug.	94	95	0.670	24	25	0.500
9 Sept.	94	98	0.030	33	37	0.080
1992						
6 May	84	85	0.660	27	26	0.880
14 May	92	93	0.680	30	33	0.440
28 May	96	96	0.830	53	56	0.460

^aNumber of plants with stem rust in a sample of 100 E+ and 100 E- plants.

^bSignificance value of the *t* test for the difference between the number of diseased E+ plants and the number of diseased E- plants.

^cStem rust severity was assessed on the modified Cobb scale.

^dSignificance value of the *t* test for the difference between the mean percent stem rust in E+ plants and the mean percent in E- plants.

observation date. Data were analyzed separately for each year. Means for E+ and E- plants on each observation date were compared by the least-squares *t* test.

RESULTS AND DISCUSSION

Stem rust occurred in susceptible plants throughout the field plots in both years and produced ragged lesions with epidermal fragments at the margins. Despite a serious epidemic in 1992, some plants remained free of the disease and were considered resistant. Pustules were produced abundantly on leaf blades and sheaths, on seed stems (when present), and on primary and secondary branches of the panicle.

In 1991, stem rust was first observed on 1 August. By 9 August, sufficient stem rust had developed that an assessment of percent infection could be made for each plant. Assessments were made again on 19 August, 23 August, 29 August, and 9 September. Because of a lack of vernalization, the stage of plant growth was variable within the stand and was not considered in the assessment. In 1992, stem rust severity was assessed on 6 May (90% head emergence), 14 May (100% anthesis), and 29 May (early dough stage).

Stem rust severity in E+ and E- plants of Kentucky 31 increased over time in each year (Table 1). In both years and on each assessment date, stem rust severity in E+ plants was similar to that in E- plants. The number of E+ plants free of the disease was also similar to the number of rust-free E- plants, and only on the first and last assessment dates were the means of E+ plants significantly

different from those of E- plants. The means of E+ and E- plants on 1 August and 9 September 1991 were different in opposite directions. Based on these data, infection of tall fescue by *A. coenophialum* does not appear to affect stem rust severity under the conditions of this experiment, nor is stem rust resistance in plants of Kentucky 31 influenced by endophyte infection. These results are consistent with our earlier report (17) that the presence of *A. coenophialum* in tall fescue seedlings does not influence the stem rust infection type resulting from inoculation with *P. g. graminicola*.

This study and the study with seedlings inoculated under controlled conditions (17) show that gene expression for resistance or susceptibility in the host and for virulence or avirulence in the pathogen is not influenced by the presence of this endophyte in tall fescue. On the basis of these results and the results of others (5,6), generalizations about increased (or induced) resistance to plant pathogens by endophyte infection of cool-season grasses would not seem justified. It has also been shown that different clones of E+ tall fescue respond differently in several physiological responses (1). Apparently, specific host-endophyte interactions produce very specific host responses, and not all E+ plants respond the same way to pathogens.

ACKNOWLEDGMENT

We thank Lisa Deppmeier, Esther Fleming, and Craig McNeal for technical assistance in this study.

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