

Reaction of Tall Fescue Infected and Noninfected by *Acremonium coenophialum* to *Puccinia graminis* subsp. *graminicola*

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ABSTRACT

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Seedlings of the tall fescue cultivar Kentucky 31 infected or not infected by the endophyte *Acremonium coenophialum* were evaluated for reaction to *Puccinia graminis* subsp. *graminicola* in the greenhouse. Seedlings (11 wk old) were rated for rust infection type (0-4 scale) 2 wk after inoculation with urediniospores. Seedlings rated 0 or 1 were considered resistant and those rated >1, susceptible; 27% of the infected and 19% of the noninfected seedlings were resistant. In a second experiment, 14 seedlings (10 wk old) each of 20 tall fescue cultivars were inoculated and rated; 12 were infected by endophyte and eight were not. Among the infected cultivars, 7% of the seedlings were rated infection type 0, 2% type 1, 7% type 2, 21% type 3, and 63% type 4. Among the noninfected cultivars, 7% of the seedlings were rated infection type 0, 1% type 1, 4% type 2, 17% type 3, and 71% type 4. Based on these observations, the presence of *A. coenophialum* in seedlings did not influence infection type of *P. g. graminicola*.

Tall fescue (*Festuca arundinacea* Schreb.) is an important forage and pasture grass grown on about 14 million hectares in the southeastern United States (5). It grows well during cool winters, survives warm summers, and tolerates soils with poor drainage or low pH. It also produces a heavy turf, making it suitable for lawns and stabilization of road banks.

A fungal endophyte, *Acremonium coenophialum* Morgan-Jones & Gams (19), that grows intercellularly in the foliage of tall fescue is responsible for a fescue toxicity syndrome in cattle (3). *A. coenophialum* produces alkaloids that are toxic to cattle (2), and steer performance may be influenced by interseeding clover into tall fescue pastures to dilute toxic effects (17).

A beneficial aspect of endophyte infection in tall fescue is that infected plants are more resistant to insect attack than infection-free plants (25). Endophyte infection in tall fescue also confers drought tolerance and greater persistence (6,22, 30). In greenhouse experiments (8), the endophyte enhanced the rate of seed germination, seedling vigor, and seed set. In field experiments (20), however, in-

creasing 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 among endophyte-infected grasses and plant pathogens have been reported to reduce disease severity (11,12,14,31), reduce nematode reproduction rates (13,21,30), or have no effect on pathogenesis or sporulation (10).

These biological and edaphic interactions have stimulated the development and release of endophyte-free cultivars of tall fescue for hay and pasture use. Tall fescue plants that are dark green and have narrow leaf blades and slow growth rates have been selected to develop turf-type cultivars for amenity grasses. Several of the turf-type cultivars are infected by *A. coenophialum*. Demand for certified seed of both forage- and turf-type cultivars of tall fescue has increased. Production of certified seed in Oregon increased from 4,654 ha in 1981 (18) to 27,180 ha in 1989 (R. Cook, *personal communication*).

In 1987, stem rust, caused by *Puccinia graminis* Pers.:Pers. subsp. *graminicola* Z. Urban, was found for the first time in tall fescue in the Willamette Valley of Oregon (28). Because of the economic importance of tall fescue, cultivars were evaluated in the greenhouse for resistance to stem rust (26). No cultivars were resistant, but individual plants were found either with no or with few and small uredinia and were considered resistant.

The present study was undertaken to evaluate effects of the endophyte on development of stem rust in tall fescue. Stem rust uredinia infection types were

evaluated in endophyte-infected and endophyte-free cv. Kentucky 31 and in 20 cultivars of forage- or turf-type cultivars of tall fescue. A brief summary of this research was presented elsewhere (27).

MATERIALS AND METHODS

Experiment No. 1. Seeds from endophyte-infected and endophyte-free 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 (9). Seeds were rinsed between and after treatments several times with sterile deionized water to remove acid and chlorine residues. Excess water was drained off and 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 ($50 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 8 hr at 25 C and 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*. Forty-nine endophyte-free (EF) and 49 endophyte-infected (EI) seedlings were transplanted into single cone-shaped plastic containers (3.8×21 cm) containing fine-grade vermiculite. Cones containing EF and EI 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 plants were rooted well. The racks were then moved to a greenhouse at 20 ± 5 C for 3 wk. Plants were watered daily and fertilized weekly with N-P-K (23:18:16, Ferti-lome) to maintain vigorous growth. Plants were placed under high-intensity light ($600 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) 24 hr before inoculation. The study was repeated twice.

Experiment No. 2. Foundation seed received from breeders of 20 cultivars was stored in sealed containers at 4 C until a 2-g subsample was taken to determine percent endophyte infection in each cultivar. Seeds were digested in 5% NaOH and stained with aniline blue in lactophenol, then 50 seeds were examined at $\times 160$ or $\times 400$ for coiled and convoluted mycelium typical of *A. coenophialum* (7,29). Percent endophyte infection for each cultivar was determined by the number of seeds infected.

Seedlings of each cultivar were established from seeds germinated on blotter

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paper moistened with 0.1% KNO₃ and incubated in a germination chamber with alternating cycles of 25 C with 16 hr light (50 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and 15 C with 8 hr of dark. After 2 wk, 14 individual seedlings for each cultivar were transplanted in single cone-shaped plastic containers and rooted as previously described. Seedlings were grown in a chamber with alternating cycles of 20 C with 16 hr of light (489–501 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and 15 C with 8 hr of dark for 3 wk. Leaves were cut back to a length of 2–3 cm and returned to the greenhouse (20 \pm 5 C) for 2 wk, then moved to a growth chamber for 3 wk. Seedlings were 10 wk old when inoculated with urediniospores of stem rust. Cultivars were replicated five times in a randomized complete block design.

Stem rust inoculum. Urediniospores of stem rust were collected in June 1989 from plants of tall fescue cv. Bonanza growing in a field near Corvallis, Oregon. The culture was maintained or increased on plants of Bonanza growing in a greenhouse at 20 \pm 5 C.

Fresh urediniospores were collected from 14- to 21-day-old sporulating pustules into a No. 00 gel capsule with a vacuum cyclone microcollector (4) and suspended in Soltrol 170, a highly refined, nonphytotoxic oil frequently used in rust inoculations of cereal grasses that does not influence percent germination of urediniospores, host penetration, or number of infections (24). Germinability of urediniospores was determined before inoculation by either dusting them on the surface of 2% water agar or by spreading a Soltrol 170 suspension on the surface of water agar and allowing Soltrol 170 to evaporate. Urediniospores were incubated in the dark for 16–18 hr at 18 C. Germination ranged from 40 to 84%.

Inoculation and infection. Urediniospores ($4.5\text{--}11.7 \times 10^6$ per milliliter) suspended in Soltrol 170 were applied uniformly to upper and lower leaf surfaces of individual plants with a spore-oil atomizer (4). Each rack of plants received 2–2.5 ml of the urediniospore suspension. After oil residue evaporated, seedlings were placed in a dew chamber without light at 18 C for 16–18 hr, followed by light (intensity front to back of the dew chamber ranged from 110 to 30 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at 25 C for 4–6 hr. Halfway through the light cycle, plant racks were rotated 180° to provide light to two sides of plants. This procedure was used to inoculate plants in both studies.

Incubation. After 20–24 hr periods of infection, racks of seedlings in the EI and EF Kentucky 31 study were placed in plastic-lined wooden boxes 5 cm high and returned to the greenhouse (20 \pm 5 C). Seedlings in the 20-cultivar study were also placed in 5-cm-high boxes and moved into a growth chamber providing 16 hr of light (489–501 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at

25 C and 8 hr of dark at 20 C. Plants were watered daily from below to avoid wetting the foliage. During the incubation period, seedling growth was vigorous, but only inoculated leaves were rated for stem rust.

Infection types. Plants were examined daily beginning 6–7 days after inoculation. Infection type for each plant was rated 14 days after inoculation by a 0–4 system used to rate stem rust infection type in cereal grains, except that the “fleck” and the mesothetic reaction classes were omitted (23). Seedlings rated 0 or 1 were considered resistant and those rated 2, 3, or 4 were considered susceptible. In the first repetition of the first experiment, stem rust severity was rated in EF and EI Kentucky 31 according to a modified Cobb scale (0 = trace, to 5, 10, 20, 40, 60, or 100% infection) (24).

Data analysis. Spearman’s rank correlation was used in experiment No. 1 to compare the number of EI and EF Kentucky 31 seedlings in the five stem rust infection types. In experiment No. 2, the number of seedlings in each infection type category (0–4) were totaled and used to compare cultivars using a multivariate factor analysis as described by Manly (16) and applied by Abernathy et al (1).

RESULTS AND DISCUSSION

In both experiments, seedling growth was vigorous during the incubation period. Pustules erupted 7–9 days after inoculation, and uredinia developed only on inoculated leaves. No secondary spread of inoculum occurred in either experiment. Attempts to rate stem rust severity using the modified Cobb scale were unsuccessful because vigorous leaf elongation interfered with evaluation. Data on stem rust severity collected for seedlings in the first run of experiment No. 1 were not reported.

Stem rust infection type scores for the seedlings in both repetitions of experiment No. 1 were combined. The distribution of infection types was similar for both EI and EF Kentucky 31 seedlings (Table 1). Infection types for both EI and EF seedlings ranged from resistant (rated 0 or 1) to susceptible (rated 2, 3, or 4). Results were similar with the EI and EF Kentucky 31 seedlings obtained from

foundation seed and evaluated in experiment No. 2 (Table 1).

The numbers of EI and EF Kentucky 31 seedlings in each of the five infection types were compared by Spearman’s coefficient (r_s) of rank correlation. With seedlings rated in experiment No. 1, the correlation between EI and EF plants was 0.98 ($P < 0.01$). For Kentucky 31 seedlings in experiment No. 2, the correlation between EI and EF plants was 0.90 ($P < 0.05$).

In the experiment No. 2, seeds of 12 of the cultivars contained *A. coenophialum* at levels ranging from 2 to 86% (Table 2). The remaining eight cultivars—Adventure, AU Triumph, Cimmaron, Fawn, Finelawn I, Forager, noninfected Kentucky 31, and Maximize—were endophyte-free.

Considerable variation was found among cultivars for the number of seedlings in each infection type. However, infection by *A. coenophialum* did not appear to affect stem rust infection type. For example, cultivar G1-307 had the highest percentage of endophyte-infected seeds and all 70 seedlings tested were susceptible to stem rust, whereas cvs. Fawn and Cimmaron, both endophyte-free, were also highly susceptible to rust. Conversely, of the six cultivars with the most resistance to stem rust, four (Arid, infected Kentucky 31, Mesa, and Short-stop) had 32–84% endophyte-infected seeds and two were endophyte-free (Finelawn I and noninfected Kentucky 31).

The numbers of seedlings in the five classes of stem rust infection types in the 12 cultivars infected by the endophyte were used in a factor analysis. Five factors were identified (Table 3). Endophyte level was the predominant component (0.984) of the second factor and was not associated with any other factors. Results of these analyses indicate that the distribution of stem rust infection types within the 12 cultivars was independent of endophyte infection level.

Individual plants of the 20 cultivars rated for stem rust infection types were not tested for endophyte; the time required for staining and examining tissue from each plant would be prohibitive. Consequently, the percentage of plants with endophyte may not be the same as the percentage of seeds with endophyte

Table 1. Distribution of infection type^a categories for *Puccinia graminis* subsp. *graminicola* in endophyte-infected and endophyte-free plants of tall fescue cv. Kentucky 31

| Experiment | No. of plants per rust infection type | | | | | Total |
|--------------------|---------------------------------------|----|---|----|----|-------|
| | 0 | 1 | 2 | 3 | 4 | |
| No. 1 | | | | | | |
| Endophyte-infected | 16 | 10 | 5 | 18 | 49 | 98 |
| Endophyte-free | 11 | 8 | 8 | 24 | 47 | 98 |
| No. 2 ^b | | | | | | |
| Endophyte-infected | 8 | 1 | 6 | 10 | 45 | 70 |
| Endophyte-free | 17 | 1 | 4 | 11 | 37 | 70 |

^aAfter Roelfs (23).

^bAdditional data in Table 2.

Table 2. *Puccinia graminis* subsp. *graminicola* infection type scores for seedlings of 20 cultivars of tall fescue

| Cultivar | Percent endophyte | Rust infection type ^a | | | | | Total |
|---------------------------------|-------------------|----------------------------------|---|----|----|----|-----------------|
| | | 0 | 1 | 2 | 3 | 4 | |
| GI-307 | 86 | 0 | 0 | 0 | 1 | 69 | 70 |
| Kentucky 31, endophyte-infected | 84 | 8 | 1 | 6 | 10 | 45 | 70 |
| Finelawn 5GL | 76 | 2 | 3 | 6 | 22 | 37 | 70 |
| Arid | 46 | 16 | 2 | 2 | 13 | 37 | 70 |
| Mesa | 46 | 10 | 4 | 11 | 13 | 32 | 70 |
| Apache | 40 | 5 | 2 | 4 | 13 | 46 | 70 |
| Shortstop | 32 | 8 | 3 | 5 | 27 | 27 | 70 |
| Thoroughbred | 28 | 6 | 1 | 7 | 13 | 43 | 70 |
| Monarch | 16 | 1 | 0 | 9 | 8 | 52 | 70 |
| Rebel II | 14 | 3 | 1 | 5 | 19 | 42 | 70 |
| Bonanza | 10 | 1 | 0 | 2 | 27 | 39 | 69 ^b |
| Johnstone | 2 | 1 | 1 | 0 | 10 | 57 | 69 ^b |
| Adventure | 0 | 2 | 1 | 4 | 12 | 51 | 70 |
| AU Triumph | 0 | 0 | 1 | 4 | 11 | 53 | 69 ^b |
| Cimmaron | 0 | 0 | 0 | 7 | 13 | 50 | 70 |
| Fawn | 0 | 0 | 1 | 0 | 12 | 57 | 70 |
| Finelawn I | 0 | 10 | 2 | 4 | 13 | 39 | 68 ^b |
| Forager | 0 | 5 | 0 | 0 | 5 | 60 | 70 |
| Kentucky 31, endophyte-free | 0 | 17 | 1 | 4 | 11 | 37 | 70 |
| Maximize | 0 | 2 | 1 | 1 | 17 | 48 | 69 ^b |

^aAfter Roelfs (23).

^bTotal reflects seedlings that died during the study from causes other than stem rust.

Table 3. Orthogonal rotated factor loadings^a from principal component factor analysis describing relationship of endophyte infection and stem rust infection type^b on 20 cultivars of tall fescue

| Predictor | Factor | | | | |
|-----------------------------------|--------|-------|--------|--------|--------|
| | 1 | 2 | 3 | 4 | 5 |
| Infection type 3 | 0.985 | | | | |
| Infection type 1 | 0.281 | | 0.261 | | 0.880 |
| Infection type 4 | -0.701 | | -0.519 | -0.355 | -0.335 |
| Endophyte infection | | 0.984 | | | |
| Infection type 0 | | | 0.978 | | |
| Infection type 2 | | | | 0.973 | |
| Sum of squares of predictors | 1.554 | 1.021 | 1.306 | 1.129 | 0.988 |
| Amount of variation explained (%) | 25.9 | 17.0 | 21.8 | 18.8 | 16.5 |

^aRotated loadings are regression coefficients for predicting variables from factors.

^bAfter Roelfs (23).

in the 50-seed sample. Also, the seed assay did not distinguish viable from nonviable endophyte. Efforts were made, however, to maintain viable endophyte in the foundation seed of each cultivar by storing seeds at 4 C in sealed plastic bags until their use in the experiment.

Infection type for cereal rusts is a phenotypic expression of the symbiotic interaction of the genotypes of the symbionts (i.e., the genotype of the host and the genotype of the pathogen) (15). This should also be true for grass rusts. Data obtained in these experiments indicate that gene expression of neither host nor pathogen is influenced by the presence of endophyte mycelium in host leaf tissue. Our data support the observations of the neutral interaction of the *Lolium* endophyte and *Drechslera dictyoides* (Drechs.) Shoemaker in *L. perenne* L. (10). Our data differ from reports of others that *Acremonium* endophytes in grasses increase disease resistance (11,12,14,31). The larger rating with crown rust, caused by *Puccinia coronata* Corda,

reported in endophyte-free than in endophyte-infected Kentucky 31 plants (11) may be due to variation in disease scoring methods (infection type vs. percent leaf rusted) or initial source of inoculum (controlled vs. noncontrolled source), the number of plants evaluated, or differences in plant stresses during infection or incubation.

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