Assessment of Resistance to *Rhizoctonia solani* in Tall Fescue Based on Disease Progress and Crop Recovery

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ABSTRACT

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Cultivars of turf-type tall fescue were assessed, in a controlled environment and in the field, for resistance to *Rhizoctonia solani*, the cause of brown patch or Rhizoctonia blight. Assessments were based on areas under disease progress curves (AUDPC) and AUDPC plus areas under crop recovery curves (AUCRC). Values of AUDPC were calculated from estimates of percent necrotic foliage during epidemics of brown patch with durations of 3-8 wk in the field or 8 days in a growth chamber. Values of AUCRC were calculated from postepidemic estimates of percent necrotic foliage over 4 wk in a greenhouse or 4-8 wk in the field. Thirty-five cultivars were separated into two groups (highly susceptible or moderately susceptible) on the basis of AUDPC values derived from epidemics of brown patch in the growth chamber. The ranking of cultivars according to AUDPC in the growth chamber was not significantly correlated with rankings in the field in 1990 ($r = 0.31$, $P = 0.17$) or 1991 ($r = 0.58$, $P = 0.09$). On the basis of relative susceptibility of cultivars to *R. solani*, however, correlation coefficients between results from the growth chamber vs. the field were $r = 0.75$, $P = 0.001$ and $r = 0.62$, $P = 0.07$ in 1990 and 1991, respectively. In all the studies, rankings of cultivars based on AUDPC were significantly ($P \leq 0.05$) correlated with rankings based on AUDPC + AUCRC.

Tall fescue (*Festuca arundinacea* Schreb.) is a widely grown turfgrass in the United States. The species is in particular demand as a turf for residential lawns because of its tolerance to heat, cold, drought, and low fertility (2). However, susceptibility to *Rhizoctonia solani* Kühn, the cause of brown patch or Rhizoctonia blight (7), continues to be a major factor limiting successful growth of this turfgrass in the southeast. *R. solani* incites foliar necrosis and crown rot in tall fescue, resulting in patches of necrotic turf that may exceed 1 m in diameter (4). Integrated methods for control of brown patch include applications of fungicides in conjunction with reductions in shade and increases in air circulation to decrease the duration of leaf wetness (18). No commercial cultivar of tall fescue is highly resistant to *R. solani*, but assessments of the severity of brown patch in field plots have revealed significant differences in foliar necrosis among populations (6). This suggests that a specific screening procedure may be useful in selecting genotypes or cultivars for reduced susceptibilty to *Rhizoctonia*.

Inoculation of tall fescue with isolates of *R. solani* does not result in hypersensitive reactions that can be used to select resistant genotypes (Burpee, *unpublished*). Therefore, in this study a screening method was designed to assess the progress of brown patch over time. It was hypothesized that specific cultivars may be characterized as "slow-blighters,"

relative to other cultivars, by analyzing the dynamics of disease progress curves. Similar screening procedures have been used with other pathosystems (8,14,22). In addition to disease progress, observations of tall fescue suggest that specific cultivars "recover" (i.e., redevelop vegetatively) faster and more completely than other cultivars after epidemics of brown patch (Burpee, *unpublished*). Therefore, the concept of crop recovery (13), i.e., an estimate of postepidemic vegetative regrowth of foliage or other plant parts, was used to assess cultivars. Selection of cultivars that show enhanced crop recovery may reveal a tolerance to *R. solani* that would be difficult to detect in a crop such as turfgrass where yield reduction is not an important result of disease.

MATERIALS AND METHODS

Studies in controlled environments. Epidemics of brown patch were induced in 35 cultivars of tall fescue (Table 1) in a growth chamber. The cultivars were grown from seed in a greenhouse in 10.2-cm-diameter Styrofoam cups containing granular calcined clay (Turface, Applied Industrial Materials Corp., Deerfield, IL). Each cup was seeded at a rate equivalent to 244 kg/ha and then watered daily and fertilized weekly with a solution containing 434, 99, and 373 ppm of N, P, and K, respectively. The turf was cut weekly to a height of approximately 6.5 cm.

Inoculum was prepared by culturing an isolate (R42) of *R. solani* AG-1 from tall fescue for 3 wk at 23 C on autoclaved ryegrain (3). The grain was then air-dried for 12 hr in a laminar-flow transfer hood, ground in a Wiley mill, and sieved to

a particle size of 1-3 mm in diameter. When 8-wk-old, turf was inoculated by placing 1.5 g of the infested grain within the turf canopy in the center of a cup. Four cups of each cultivar were treated with inoculum, and one cup of each served as a noninoculated control. Immediately after inoculation, the turf foliage was misted with water to runoff, and the cups were arranged in a completely randomized design in an acrylic mist chamber constructed within a plant growth chamber. The turf was incubated under an environmental regime of 100% RH with 14 hr light (photon flux density = $350 \mu E \cdot m^{-2} \cdot s^{-1}$) at 30 C and 10 hr dark at 24 C. At intervals of 1, 2, 4, and 8 days postinoculation, one cup of each cultivar was removed from the mist chamber and placed on a greenhouse bench, where the turf foliage dried for 24 hr. Drying resulted in greater visual contrast between necrotic and non-necrotic foliage, which facilitated estimates of disease severity (percent necrotic foliage per cup) by the Horsfall-Barratt rating scale (10). Disease severity values × time were used to prepare a disease progress curve for each cultivar. An epidemic was repeated four times for each cultivar to provide five replications for statistical analysis. The experiment was repeated once.

Crop recovery (the postepidemic regrowth of symptomless foliage) was assessed on the cups of turf that were incubated in the mist chamber for 8 days and then transferred to a greenhouse bench for 4 wk. During this period, the turf was cut with scissors to a height of 6.5 cm at weekly intervals. The turf was irrigated and fertilized by placing the cups in flats of water or, at weekly intervals, a solution containing 474, 99, and 373 ppm of N, P, and K, respectively. Water and fertilizer contacted roots through drainage holes in the bottoms of the cups; this form of irrigation limited further disease development by preventing foliar wetness. Crop recovery was assessed 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 (10). Necrosis declined in most cultivars as a result of the growth of symptomless foliage from meristems that survived the epidemics. Values of percent necrosis × time were used to prepare crop recovery curves for each cultivar-replication combination. A crop recovery curve was an extension of a disease progress curve with

an origin at the point where disease progress ceased. In this study, that point was considered to be within an hour after removal of a cup from the mist chamber, when leaf wetness became limiting for disease development. Areas under disease progress curves (AUDPC) and crop recovery curves (AUCRC) were calculated with the formula $\Sigma[(y_i + y_{i+1})/2][t_{i+1} - t_i]$, where $i = 1,2,3,...,n-1$, y_i is the amount of disease (necrosis), and t_i is the time of the ith rating (17).

Field studies. To determine correlations between results obtained in the greenhouse and observations made in the field, the severity of brown patch was assessed in 1990 and 1991 in plots of cultivars of tall fescue grown on a Cecil sandy loam (pH = 6.7) at the University of Georgia Agricultural Experiment Station (Georgia Station) in Griffin. The cultivars assessed in 1990 (Table 2) were established from seed in September 1987 at a rate of approximately 342 kg/ha in plots (1.5 × 1.5 m) arranged in a randomized complete block design with three replications. The turf was maintained at a height of 7 cm by mowing once or twice a week, irrigated as required, and fertilized with approximately 244 kg/ha of N and K and 98 kg/ha of P divided equally among five applications per year. The turf was not artificially inoculated with *R. solani*.

Brown patch was first observed in the plots in early July 1990, and visual estimates of disease severity were made on 16, 23, and 30 July and 6 August using the Horsfall-Barratt rating scale (10). Disease severity values × time were used to prepare disease progress curves for each cultivar-replication combination. On 7 and 21 August, the plots were sprayed with the fungicide iprodione (Chipco 26019 23.3F) at a rate of 3.1 kg a.i./ha in 700 L/ha of water to suppress further development of brown patch. The turf plots were rated for recovery on 14, 21, and 28 August and 4, 11, and 18 September by visually estimating the percent area of necrotic foliage and/or gaps in the foliar canopy within each plot. These disease ratings × time were used to produce crop recovery curves for each cultivar-replication combination. Values of AUDPC and AUCRC were calculated as described for the controlled environment study.

In 1991, disease assessments were made on eight cultivars of tall fescue (Table 3), including five highly susceptible and three moderately susceptible cultivars based on values of AUDPC derived from results of the controlled environment study. Plots of the cultivars were established from sod produced in a greenhouse by seeding the cultivars at a rate of 342 kg/ha on 11 February 1991 in plastic trays (46 cm long × 38 cm wide × 8 cm deep) containing Turface. The turf was watered daily, fertilized as described for the controlled environment studies, and mowed weekly to 6.5 cm with a rotary lawn mower. On 8 April 1991, the sod in each flat, each flat representing a treatment plot, was placed in a randomized complete block design with six replications on tilled Cecil sandy loam at the Georgia Station. The sod was irrigated daily, fertilized every 4 wk with 25-4-10 (N-P-K) at a rate of 49 kg N/ha, and mowed weekly with a rotary lawn mower to 6.5 cm.

The turf was inoculated on 8 July by dispersing by hand approximately 7 g of autoclaved grain infested with *R. solani* AG-1 (isolate R42) into the turf canopy of each plot. The inoculum was prepared as described for the controlled environment study. The turf was irrigated daily at 2000 to provide nightly leaf wetness for disease development. The Horsfall-Barratt rating scale (10) was used to estimate the intensity of brown patch in each plot on 21, 25, and 28 June; 1, 5, 8, 11, 15, 19, 25, and 29 July; and 8 August. On 8 August, the plots were sprayed with iprodione as described for the 1990 field trials. Crop recovery was estimated on 13 and 28 August; 4, 11, 20, and 30 September; and 8 October as described for the 1990 field trials. Calculations of AUDPC and AUCRC were made as described for the controlled environment study.

Table 1. Area under the disease progress curve (AUDPC) and AUDPC + area under the crop recovery curve (AUCRC) for epidemics of brown patch on 35 cultivars of tall fescue in a controlled environment[w]

Cultivar	AUDPC	AUDPC + AUCRC[x]
Twilight	659.8 a[y]	3,170.9 a[y]
Shortstop	614.8 a	2,905.9 a
Bonsai Dwarf	608.8 a	2,958.4 a
Mojave	555.9 a	3,334.3 a
Taurus	549.8 a	2,684.7 a
PX-3	538.6 a	2,701.0 a
Mesa	506.1 a	2,501.0 a
JB-2	502.6 a	2,454.3 a
Pacer	494.4 a	3,133.7 a
Murietta	491.6 a	3,136.9 a
Houndog	471.6 a	2,237.5 b
Adventure	467.7 a	2,790.4 a
Trident	466.4 a	3,283.5 a
Crossfire	463.1 a	1,836.9 b
Monarch	450.2 b	3,039.8 a
Penngrazer	448.3 b	2,488.8 a
Mustang	446.3 b	2,108.1 b
Finelawn I	428.3 b	2,525.7 a
Rebel Jr.	423.3 b	2,128.7 b
Falcon	422.4 b	1,982.2 b
Olympic	414.9 b	1,909.7 b
Arid (no endophyte)[z]	414.4 b	2,512.8 a
Silverado	413.8 b	2,613.1 a
DBC	412.9 b	2,921.6 a
Wrangler	407.2 b	2,026.6 b
Maverick II	405.0 b	1,533.5 b
Gala	396.3 b	1,707.7 b
Titan	386.1 b	1,712.5 b
Finelawn 5GL	365.7 b	2,028.7 b
Rebel	349.5 b	2,160.6 b
Tribute	345.8 b	2,210.6 b
Arid (endophyte)[z]	336.4 b	1,925.3 b
Bonanza	321.4 b	1,632.4 b
Apache	317.0 b	1,700.8 b
Rebel II	315.2 b	1,241.3 b

[w] Eight-week-old turfgrass was inoculated with *Rhizoctonia solani* and incubated for 1, 2, 4, or 8 days in a mist chamber with 14 hr of light per day and a light/dark temperature regime of 30/24 C.
[x] After 8 days of incubation in a mist chamber, cups of turf were placed in a greenhouse and assessed for growth of symptomless foliage at 1, 2, and 4 wk postincubation.
[y] Within a column, values followed by the same letter are not significantly different at $\alpha = 0.05$ according to the Scott-Knott cluster analysis procedure (16).
[z] Established from seed either infested (endophyte) or not infested (no endophyte) with *Acremonium coenophialum*.

Table 2. Area under the disease progress curve (AUDPC) and AUDPC + area under the crop recovery curve (AUCRC) for epidemics of brown patch on 21 cultivars of tall fescue in field plots at Griffin, Georgia, in 1990

Cultivar	AUDPC	AUDPC + AUCRC
Twilight	1,170.0 a[z]	6,636.2 a[z]
Taurus	904.9 a	6,104.8 a
Shortstop	765.4 a	5,921.2 a
Adventure	568.5 b	1,878.1 b
Monarch	434.5 b	3,017.0 a
Trident	415.4 b	2,356.3 b
Crossfire	325.1 b	2,906.1 b
Arid (endophyte)	295.0 b	1,634.7 b
DBC	286.8 b	2,553.0 b
JB-2	278.6 b	1,466.6 b
Finelawn I	275.8 b	1,603.1 b
Olympic	262.2 b	1,365.3 b
Silverado	259.5 b	1,923.9 b
Rebel	212.9 b	1,181.3 b
Falcon	207.5 b	944.6 b
Bonanza	207.5 b	936.1 b
Maverick II	185.6 b	718.7 b
Tribute	163.8 b	504.2 b
Finelawn 5GL	158.3 b	489.4 b
Apache	155.6 b	499.0 b
Rebel II	139.3 b	443.9 b

[z] Within a column, values followed by the same letter are not significantly different at $\alpha = 0.05$ according to the Scott-Knott cluster analysis procedure (16).

Table 3. Area under the disease progress curve (AUDPC) and AUDPC + area under the crop recovery curve (AUCRC) for epidemics of brown patch on eight cultivars of tall fescue in field plots at Griffin, Georgia, in 1991

Cultivar	AUDPC	AUDPC + AUCRC
Twilight	56,224 a[z]	79,675 a[z]
PX-3	55,560 a	79,417 a
Rebel Jr.	55,424 a	75,356 a
Murietta	55,155 a	82,145 a
Mojave	54,129 a	75,457 a
Apache	52,803 a	69,556 b
Houndog	45,425 b	61,851 c
Maverick II	42,143 b	55,011 c

[z] Within a column, values followed by the same letter are not significantly different at $\alpha = 0.05$ according to the Scott-Knott cluster analysis procedure (16).

Data analysis. Statistical calculations were performed with Statistical Analysis Software (SAS Institute Inc., Cary, NC) procedures. Values of AUDPC and AUDPC + AUCRC were subjected to analysis of variance, and means were statistically separated by cluster analysis at $\alpha = 0.05$ (16). Values of AUCRC were not analyzed alone because the origins of the recovery curves were not equivalent for each cultivar. To assess the relationship between data collected in the controlled environment studies and data from the field studies, Spearman's rank order correlations were performed on rankings of the cultivars based on AUDPC in the greenhouse vs. their rankings in the field in 1990 and 1991. In addition, rank order correlations were performed on the ranking of the cultivars based on values of AUDPC vs. AUDPC + AUCRC for the greenhouse study and each field study.

RESULTS

Studies in controlled environments. Repetition of the experiments gave consistent results. Regression of means in each experiment with common values in the repeated experiment resulted in a regression coefficient (R^2) >0.86 and a slope of 0.9. Disease severity was not significantly different among cultivars at 1, 2, 4, and 8 days postinoculation (Table 4). However, differences among values of AUDPC were highly significant ($P = 0.0001$). On the basis of these differences, the cultivars were separated statistically into highly susceptible (AUDPC = 659.8–450.2) and moderately susceptible (AUDPC = 450.2–315.2) groups (Table 1).

The ranking of cultivars according to AUDPC was significantly correlated ($r = 0.75, P = 0.001$) with the ranking based on AUDPC + AUCRC. However, values of AUDPC + AUCRC were significantly lower for cvs. Houndog and Crossfire than for other cultivars in the highly susceptible group (Table 1) and significantly higher for cvs. Monarch, Penngrazer, Finelawn I, Arid (no endophyte), Silverado, and DBC in the moderately susceptible group.

Field studies. There were no significant differences in AUDPC among 18 of 21 cultivars evaluated in field plots in 1990. However, a significantly higher AUDPC was associated with cvs. Twilight, Taurus, and Shortstop than with the other cultivars tested (Table 2). Values of AUDPC + AUCRC were significantly higher for cvs. Twilight, Taurus, Shortstop, and Monarch than for the other cultivars tested (Table 2). In 1991, significantly lower values of AUDPC were associated with cvs. Houndog and Maverick II and significantly higher values of AUDPC + AUCRC were associated with cvs. Twilight, PX-3, Rebel Jr., Murietta, and Mojave than for the other cultivars tested (Table 3).

The rankings of cultivars based on AUDPC in the controlled environment study were not significantly correlated with rankings in the field in 1990 ($r = 0.31, P = 0.17$) or 1991 ($r = 0.58, P = 0.09$). In 1990, however, seven of 10 cultivars with the highest values of AUDPC in the field were classified as highly susceptible in the controlled environment study. In 1991, four of five cultivars with the highest AUDPC values were classified in the highly susceptible group. In both years, the rankings of cultivars according to values of AUDPC were significantly correlated ($P < 0.05$) with rankings based on AUDPC + AUCRC ($r = 0.95$ and 0.67, respectively).

DISCUSSION

Differences in susceptibility among cultivars of tall fescue to *R. solani* were detected by analyzing AUDPC. Analysis of AUDPC, which has been used to identify disease resistance in other hosts (8,14,22), was particularly effective in detecting differences in susceptibility that were not detected by analyzing disease intensity at specific times (i.e., 1, 2, 4, or 8 days postinoculation). This is probably a result of the averaging of disease severity values in the calculation of AUDPC, which reduces variation and is reflected in a low error term relative to mean square in the analysis of variance.

The low correlation between rankings of cultivars in controlled environment and rankings in the field suggests that the AUDPC evaluations conducted in the greenhouse do not accurately reflect performance of cultivars under field conditions. In the field, however, seven of the 10 most susceptible cultivars in 1990 and four of the five most susceptible cultivars in 1991 were also identified as highly susceptible in the controlled environment study. This apparent inconsistency in results is best explained by the fact that Spearman's rank order correlations reflect the precise ranking of cultivars from the highest to the lowest value of AUDPC rather than a ranking based on relative susceptibility to *R. solani*. For

example, if the highly susceptible cultivars in Table 1 (AUDPC = 659.8–463.1) are arbitrarily assigned a value of 1 and the moderately susceptible cultivars (AUDPC = 450.2–315.2) are assigned a value of 2, Spearman's correlation coefficients for rankings of cultivars in the greenhouse vs. the field in 1990 and 1991 are $r = 0.75, P = 0.0001$ and $r = 0.62, P = 0.07$, respectively. This indicates that the methods employed in the controlled environment were effective in predicting relative susceptibility of tall fescue to *R. solani* under field conditions.

The significant correlations between the rankings of cultivars based on AUDPC vs. AUDPC + AUCRC suggest that disease progress is an accurate indicator of a cultivar's potential to produce symptomless foliage after an epidemic (i.e., to recover). However, analysis of AUDPC + AUCRC for turf grown in the controlled environment revealed that some highly susceptible cultivars (e.g., Houndog and Crossfire) showed better recovery than some moderately susceptible cultivars (e.g., Monarch, Finelawn I, and Silverado). Similar contrasts were not observed in the field, but crop recovery may still be a useful trait to assess relative tolerance of a cultivar or genotype to a pathogen in field trials.

Tolerance has been defined as the ability of a plant to endure severe disease without severe losses in yield or quality (5). When considering turfgrass, the concept of crop recovery is proposed as a measure of tolerance because, in this crop, yield is not usually an important trait, and severe disease always results in a severe loss in quality. In using this concept of tolerance to interpret the results of the 1990 field trial, cv. Monarch, which did not differ significantly from cv. Adventure according to AUDPC, would be considered less tolerant to *R. solani* according to AUDPC + AUCRC. Likewise, in 1991, cvs. Twilight, PX-3, Rebel Jr., Murietta, and Mojave would be considered less tolerant than cv. Apache. AUDPC + AUCRC is a novel assessment parameter that may

Table 4. Analysis of variance for severity of brown patch on 35 cultivars of tall fescue at 1, 2, 4, and 8 days postinoculation and for the area under the disease progress curve (AUDPC) for an 8-day epidemic in a controlled environment[z]

Source of variation	df	MS	F	P	CV
Cultivars at 1 day	34	1.0	0.81	0.765	52.3
Error	135	1.2			
Cultivars at 2 days	34	49.8	0.34	0.999	62.6
Error	135	145.3			
Cultivars at 4 days	34	498.1	0.76	0.817	57.0
Error	135	651.6			
Cultivars at 8 days	34	382.4	1.34	0.123	35.4
Error	135	282.3			
AUDPC	34	113,326.9	6.99	0.0001	28.2
Error	135	16,210.8			

[z] Eight-week-old turfgrass was inoculated with *Rhizoctonia solani* and incubated for 1, 2, 4, or 8 days in a mist chamber with 14 hr of light per day and a light/dark temperature regime of 30/24 C.

benefit researchers in the selection of turfgrasses, and possibly other crops, that show tolerance to necrotrophic pathogens. In the research reported here, AUCRC was not analyzed separately from AUDPC because disease progress curves did not terminate at the same level of severity for each cultivar. That is, the origins of the crop recovery curves were not equivalent. Future modifications in experimental procedures (e.g., the strategic use of fungicides) that may result in the termination of epidemics at the same level of disease severity on each cultivar could provide for the analysis of crop recovery distinct from that of disease progress.

The significantly lower value of AUDPC + AUCRC for cv. Arid infected with the endophyte *Acremonium coenophialum* Morgan-Jones & Gams than for Arid not infected with the endophyte is an example of the potential importance of crop recovery as an assessment parameter. Tall fescue infected with *A. coenophialum* has shown insect resistance (9,11,15), resistance to root-parasitic nematodes (12,20), and tolerance to drought (1,21). Disease resistance in endophyte-infected grasses has not been firmly established (19). As observed in the controlled environment study, the impact of endophyte infection on disease may be expressed as enhancement of crop recovery rather than suppression of disease progress. Unfortunately, sufficient endophyte-free seed was not available to assess this under field conditions.

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