Screening Tall Fescue for Resistance to Rhizoctonia solani and Rhizoctonia zeae **Using Digital Image Analysis**

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Abstract

Brown patch, caused by Rhizoctonia solani, is a destructive disease on tall fescue. Compared with R. solani, Rhizoctonia zeae causes indistinguishable symptoms in the field but varies in geographic distribution. This may contribute to geographic variability observed in the resistance response of improved brown patch-resistant cultivars. This study examined R. solani and R. zeae susceptibility of four cultivars, selected based on brown patch performance in the National Turfgrass Evaluation Program (NTEP), and nine plant introductions (PIs). Twenty genotypes per PI/cultivar were evaluated by using four clonal replicates in a randomized complete block design. Plants were inoculated under controlled conditions with two repetitions per pathogen. Disease severity was assessed digitally in APS Assess, and analysis of variance and correlations were performed in SAS 9.3. Mean disease severity was higher for R. solani (65%) than for R. zeae (49%) (P = 0.0137). Interaction effects with pathogen were not significant for PI (P = 0.0562) but were for genotype (P < 0.001). Moderately to highly resistant NTEP cultivars compared with remaining PIs exhibited lower susceptibility to R. zeae (P < 0.0001) but did not differ in susceptibility to R. solani (P =0.7458). Correlations between R. solani and R. zeae disease severity were not significant for either PI (R = 0.06, P = 0.8436) or genotype (R = 0.11, P = 0.09). Breeding for resistance to both pathogens could contribute to a more geographically stable resistance response. Genotypes were identified with improved resistance to R. solani (40), R. zeae (122), and both pathogens (26).

Keywords: cultivar/resistance, disease management, fungi, turf

Tall fescue (Schedonorus arundinaceus Schreb., previously Festuca arundinacea Schreb.), is a cool season turfgrass generally well adapted and widely grown in the transition zone. The transition zone is an area where both cool and warm season grasses are present but to which neither is fully adapted. In the summer, tall fescue is subject to a number of different turfgrass diseases, with brown patch, caused by the fungus Rhizoctonia solani Kuhn (synonym: Thanatephorus cucumeris [Frank] Donk), being one of the most common and destructive. Planting disease-resistant cultivars is a simple, inexpensive disease control option, and many tall fescue cultivars are available that have some level of brown patch resistance. However, the ranking of resistance response to brown patch may not be consistent across locations. This inconsistency is confirmed in data collected for the National Turfgrass Evaluation Program (NTEP), a widely used and recognized U.S. program for evaluating various turfgrass characteristics in trials performed in multiple locations (Morris 2002). Some variation may be attributed to within- and between-location differences in isolate loads and virulence, environmental conditions, and cultural practices. However, an additional contributing factor may be pathogens that cause symptoms similar to those of R. solani but are not included in screening for brown patch resistance.

Rhizoctonia leaf and sheath spot is caused by Rhizoctonia zeae Voorhees (synonym: Waitea circinata var. zeae Warcup and Talbot), a pathogen that can produce symptoms very similar to, and often indistinguishable from, those produced by R. solani (Martin and Lucas 1984). Often the two diseases can be distinguished only through examination of cultured isolates of the causal fungi. R. zeae typically is

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not included in brown patch resistance breeding programs (Bokmeyer et al. 2009a, b; Ford et al. 2001; Fraser et al. 1999; Meyer 1991; Watkins and Meyer 2004). This may result in brown patch disease resistance ratings that are unintentionally influenced by the presence or absence of R. zeae across evaluation locations.

R. solani is thought to be dominant in northern regions, whereas R. zeae is thought to be dominant in southern regions (Elliot 1999). Studies performed in New Jersey, South Carolina, and Virginia reported higher than expected frequencies of R. zeae (Martin et al. 2001; McCall 2006; Plumley 1988). In 2001, Martin et al. reported significant differences in isolation frequency of R. zeae and R. solani between two locations in South Carolina. Research by McCall (2006) reported similar results in Virginia. R. zeae was isolated from brown patch-like symptoms in Virginia and appeared to be more prevalent in the eastern half of the state. R. zeae also had a higher temperature optimum compared with R. solani, which may explain the greater incidence found at lower elevations or in areas with higher average temperatures. These differences in geographic distribution could result in what appears to be highly variable resistance response to brown patch (R. solani) but may be caused by an underlying susceptibility to R. zeae.

The objectives of this study were to use digital image analysis to determine whether the resistance response of tall fescue germplasm differs between R. solani and R. zeae and to identify tall fescue germplasm with resistance to R. solani, R. zeae, or both pathogens. Results from this study will help determine whether there is a need to include both pathogens when screening for brown patch resistance in tall fescue and identify germplasm with resistance that breeders can use when developing brown patch-resistant cultivars.

Materials and Methods

Plant materials and maintenance. Nine tall fescue plant introductions (PIs) were selected from the tall fescue collection within the National Plant Germplasm System (NPGS), which is managed by the U.S. Department of Agriculture's Agricultural Research Service (Table 1). Four commercial cultivars were chosen based on average rating for brown patch resistance across all locations in the 2002 NTEP trials for tall fescue (Morris 2002) (Table 1). In the NTEP trials, brown patch was rated on a scale of 1 to 9, with 9 indicating no disease. Cultivars selected included Kentucky 31 E+ (7.7 out of 9), Millennium (7.3 out of 9), Falcon II (6.9 out of 9), and Stetson (6.5 out of 9). The remaining PIs were selected from the NPGS based predominantly on similarity of collection location climate to eastern Virginia.

Because tall fescue is highly self-incompatible, 20 seeds, considered to represent 20 different genotypes, were arbitrarily selected from within each PI/cultivar and were individually planted in 3.8-cm diameter conetainers filled with potting media containing biofungicide (Bacillus subtilis MBI 600). Plants were maintained in a glasshouse, watered at a rate of 2.55 cm/h for 3 min daily by an overhead irrigation system, and fertilized with three applications per month of a 20-20-20 mix at an N rate of 48.8 kg/ha. Plants were cut twice a week to a height of 7.6 cm with handheld trimmers. After plants produced sufficient tillers, each genotype was divided into 16 clonal plants.

Disease treatment. Two disease screens were performed per pathogen. Each screen contained 1,040 individual plants (13 PIs × 20 genotypes × 4 replications). Plants were arranged in a randomized complete block design with two blocks per disease chamber. Because of space limitations, two chambers were needed for each disease screen.

Disease chambers were the length and width of a single greenhouse bench and consisted of a polyvinyl chloride pipe frame enclosed on all sides with opaque white plastic. The plastic on one side of the chamber was sealable with Velcro so that plants could be accessed as necessary. A heating mat set to 100°C was placed across the bottom of each chamber to increase temperature and facilitate disease development. High humidity (approximately 99%) was maintained through a mist irrigation system consisting of three irrigation heads per chamber, with tubing threaded through holes cut in the top of the chamber.

During the disease screening period, overhead irrigation was applied for 3 min each morning at a rate of 2.55 cm/h. Temperature in the chambers was 6.5 to 8.5°C higher than outside temperatures. This equated to approximately 27 to 29°C during the R. solani screen and 32.5 to 34.5°C during the R. zeae screen.

Inoculation. Three highly virulent *R. solani* isolates were used to create inoculum. They were selected from a preliminary screening of 14 R. solani isolates collected from creeping bentgrass putting greens at the Virginia Tech Turfgrass Research Center in Blacksburg, Virginia (data not shown). Three isolates of R. zeae were selected based on their performance in virulence studies by McCall (2006). They were collected from tall fescue and Kentucky bluegrass at several locations across the state of Virginia.

Inoculum was created from filter paper cut to $2 \text{ cm} \times 0.5 \text{ cm}$, autoclaved for 1 h before use, and placed radially around a 4-mm diameter plug of R. solani on potato dextrose agar (Sykes et al. 2017).

Table 1. Plant material included in Rhizoctonia solani and Rhizoctonia zeae disease screens. Millennium, Falcon II, Kentucky 31 E+, and Stetson were selected based on performance in the 2002 NTEP trials. The remaining plant introductions (PIs) were selected predominantly based on collection locations with climates similar to that of southeastern Virginia

PI/cultivar	Accession name and identifier	Collection location	Type	Notes
578712	Alta	Oregon	Cultivar	
600801	Rebel	New Jersey	Cultivar	
561431	Kentucky 31	Kentucky	Cultivar	With endophytes
561430	Kentucky 31	Kentucky	Cultivar	No endophytes
578715	Fawn	Oregon	Cultivar	
598891		Morocco	Wild material	
469244	Asheville	North Carolina	Landrace	
598945		Sardinia, Italy	Wild material	
636598		Washington	Wild material	
Millennium		C	Cultivar	
Falcon II			Cultivar	
Stetson			Cultivar	
Kentucky 31 E+			Cultivar	

Table 2. Mean disease severity (%) by cultivar/plant introduction (PI) by Rhizoctonia solani screen, Rhizoctonia zeae screen, and averaged across both pathogens. Cultivar/PIs are sorted in ascending order based on mean disease severity across pathogen screens

Cultivar/PI	Number of genotypes evaluated	R. solani screen		R. zeae screen		Across pathogens	
		Disease (%)	Low-disease genotypes (%) ^y	Disease (%)	Low-disease genotypes (%) ^y	Disease (%)	Low-disease genotypes (%) ^y
Millennium	20	62	30	38	100	50 b ^z	30
Falcon II	20	62	35	41	85	52 b	30
Kentucky 31 E+	19	69	5	39	89	54 ab	5
Stetson	20	68	5	42	75	55 ab	0
600801	20	58	35	53	40	55 ab	20
561430	19	61	21	50	47	55 ab	5
598945	15	63	13	52	40	57 ab	7
561431	18	61	17	55	22	58 ab	0
469244	16	66	25	51	50	58 ab	25
578712	20	63	15	54	25	58 ab	10
536598	20	70	10	49	40	60 ab	5
578715	20	67	0	55	25	61 ab	0
598891	15	74	0	58	0	66 a	0

y Low-disease genotypes were classified as genotypes exhibiting disease severity not significantly different from the lowest mean disease severity value within each respective screen at an alpha level of 0.05. Low-disease genotypes across pathogens were identified as genotypes that exhibited this definition of low disease in both the R. solani and R. zeae screens.

² Means were separated with Tukey's HSD. Means followed by the same letter are not significantly different between PIs at a probability level of 0.05. Because no significant interaction was indicated (P = 0.0562), mean separation is indicated only for PI disease severity averaged across both pathogens and not given for PI by pathogen.

Plates were maintained for 2 weeks to allow sufficient colonization of the filter paper. Plants in the disease chamber were inoculated by three infested filter paper pieces, one piece from each isolate, placed in the plant canopy of each conetainer. Inoculated plants were placed in the disease chamber for 12 days. Upon removal, plants were placed on subirrigated greenhouse benches for 24 h to allow the canopy to dry.

Disease evaluation. Disease evaluation was performed as described for the digital image analysis of whole plant canopy macro in Sykes et al. (2017). In summary, pictures were taken before and after disease screening. An index mark was placed on conetainers and the stand before inoculation to provide consistent leaf orientation between the before and after pictures. A digital camera was placed on a tripod with the orientation adjusted to take a top-down picture. The camera's aperture was set at f/8, with the shutter speed adjusted manually for each image to read zero on the light meter. Two light stands, each containing four 40-watt compact, semispiral, daylight balanced (5500 K) fluorescent bulbs were placed on either side of the stand to provide even illumination.

The following procedures were used to define a macro in APS Assess (APS Press) image analysis software.

- The plant area was selected from the background at a hue threshold of 31-low and 191-high. The background was replaced with a solid blue background via the command Edit > Substitute background.
- 2. The soil was selected at an intensity threshold of 66-low and 255-high. The soil was replaced with a solid blue background via the command Edit > Substitute background.

- 3. The total leaf area was selected at a hue threshold of 31-low and 191-high. The total area in pixels was calculated by selecting the "Area" button on the threshold panel.
- 4. The total lesion area was selected. Sykes et al. (2017) reported increased accuracy with a user-defined hue threshold for selecting lesions. To increase accuracy in defining disease hue thresholds, a random selection of 10 images from within each replication of each disease screen were assessed individually to determine the average hue threshold values that most accurately selected lesioned areas. The hue threshold used to identify lesions was modified within the macro to correspond with the chosen value for each replication within each screen. The hue values used for each screen to select lesions are as follows:
 - a. R. solani repetitions 1 and 2
 - i. replications 1 and 2: 31-low, 110-high ii. replications 3 and 4: 31-low, 115-high
 - b. *R. zeae* repetitions 1 and 2
 - i. replications 1 to 4: 31-low, 101-high
- Disease severity was calculated as a ratio of total lesion area to total leaf area.
- Total disease was calculated as the percentage disease of the post-inoculation picture minus the percentage disease of the pre-inoculation picture.

Statistics. All analyses were performed in SAS version 9.3. Genotypes with three replicates missing from one or more repetitions were removed from the dataset. An analysis of variance was performed via the GLIMMIX procedure to assess PI × pathogen and

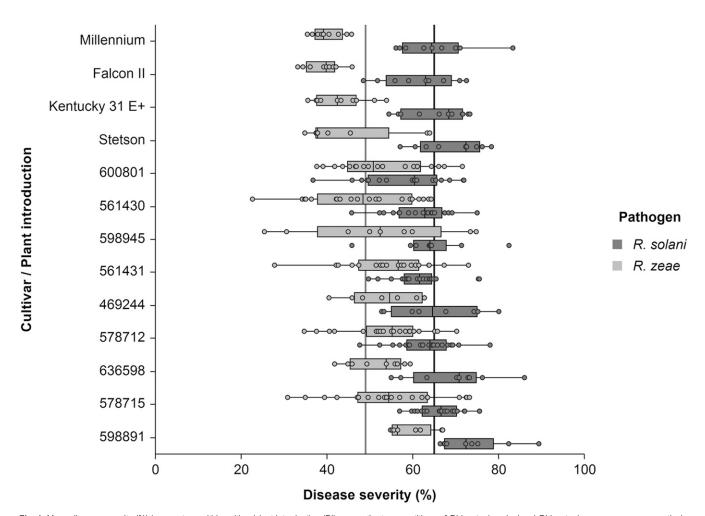


Fig. 1. Mean disease severity (%) by genotype within cultivar/plant introduction (PI) across the two repetitions of *Rhizoctonia solani* and *Rhizoctonia zeae* screens, respectively. Cultivar/Pls are sorted by mean disease severity averaged across both pathogens. Boxes and whiskers indicate interquartile range. Circles represent mean disease severity of individual genotypes within each PI. Dark gray indicates performance in *R. solani* screens, and light gray indicates performance in *R. zeae* screens. The solid dark gray line at 49% represents the mean disease severity across the *R. zeae* screens.

genotype × pathogen interactions in regard to disease severity. The following models were used:

 $Y = pathogen + PI + pathogen \times PI + repetition (pathogen)$

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+ replicate (PI*pathogen*repetition)
Y = pathogen + genotype + pathogen \times genotype
     + repetition (pathogen) + replicate
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 $(genotype \times pathogen \times repetition)$

Contrast statements were used to compare the average disease severity of cultivars selected from the NTEP trials for superior brown patch resistance to the average disease severity across the remaining PIs and cultivars for R. solani and R. zeae disease severity, respectively. Means separation via the diff=control command in proc glimmix was then used to identify genotypes that did not differ significantly from the genotype exhibiting the lowest disease severity within each respective pathogen screen at an alpha level of 0.05. Within these models, genotype, PI, and pathogen were considered fixed effects and repetition and replicate were considered random. The correlation procedure was used to determine Pearson correlation coefficients between R. solani and R. zeae disease severity by genotype and by PI.

Results

Mean disease severity did not differ significantly (P = 0.3954) between the two repetitions within each pathogen screen. Mean disease severity differed by pathogen (P = 0.0137), PI (P = 0.0373), and genotype (P < 0.0001). Mean disease severity was significantly higher in R. solani screens (65%) compared with R. zeae screens (49%) (P =0.0133).

Mean disease severity by PI ranged from 58 (PI 600801, Rebel) to 74% (PI 598891, wild material) within the R. solani screen and from 38 (Millennium) to 58% (PI 598891, wild material) within the R. zeae screen (Table 2). Across pathogens, the cultivar Millennium had the lowest disease severity (50%), and PI 598891 had the highest disease severity (66%). Disease severity was highly variable within each PI. Across genotypes, mean disease severity ranged from 37 to 86% in the R. solani screens and 23 to 74% in the R. zeae screens (Fig. 1).

The observed P value for interaction between PI and pathogen was P = 0.0562, suggesting that although R. zeae tended to cause less disease, PIs that exhibited greater resistance to R. solani also exhibited greater resistance to R. zeae. However, the interaction between genotype and pathogen was significant (P < 0.0001). Likewise, correlations between R. solani and R. zeae disease severity were weak and not significant for both PI (R = 0.11, P = 0.0949) and genotype (R = 0.06, P = 0.8436) (Fig. 2).

PI × pathogen interaction was further examined via contrast statements to determine whether the disease severity response to R. solani differed from that of R. zeae in the cultivars selected from the 2002 NTEP tests for superior brown patch resistance compared with the remaining PIs. The three cultivars selected based on superior performance in the 2002 NTEP tests (medium resistance, Falcon II; high resistance, Kentucky 31 E+ and Millennium), on average, exhibited significantly lower (P <0.0001) R. zeae disease severity compared with the average disease severity of the remaining PIs (Fig. 1). Counter to expectation, resistance to R. solani did not differ significantly (P =0.7458) between the group of superior brown patch–resistant cultivars and the remaining PIs.

Although no genotypes were found with complete resistance to either pathogen, genotypes were identified with low disease susceptibility. Genotypes with low disease susceptibility were classified as those that did not differ statistically at an alpha level of 0.05 from the genotype exhibiting the lowest disease severity within a test. The NTEP-selected cultivars contained the highest percentage of genotypes with low R. zeae disease severity (Table 2). These percentages followed the same order by which these cultivars were selected from the NTEP results. Millennium and Kentucky 31 E+, which exhibited low susceptibility to brown patch in the NTEP trials, contained the highest percentage of genotypes (100 and 89%, respectively) with low R. zeae disease susceptibility. Falcon II, which was ranked in the middle for brown patch susceptibility, exhibited a slightly lower percentage of genotypes with low R. zeae disease susceptibility (85%). Stetson, which had higher brown patch susceptibility in the NTEP trials, contained the lowest percentage of genotypes exhibiting low *R. zeae* susceptibility (75%) among the four cultivars. All four of these cultivars had much higher percentages of genotypes exhibiting low R. zeae susceptibility compared with the remaining

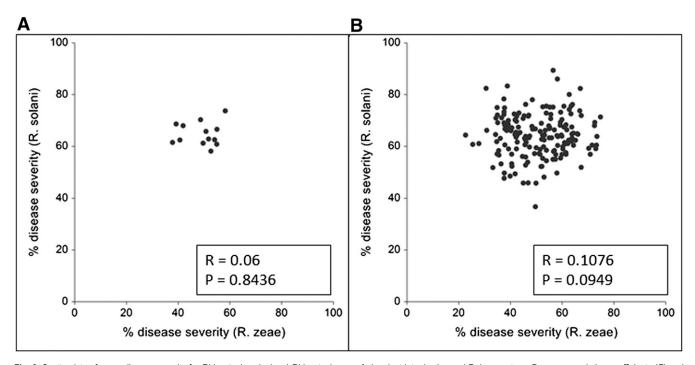


Fig. 2. Scatterplots of mean disease severity for Rhizoctonia solani and Rhizoctonia zeae A, by plant introduction and B, by genotype. Pearson correlation coefficients (R) and associated P values are given for each graph.

PIs. The percentage of genotypes exhibiting low R. zeae susceptibility within the remaining PIs ranged from 22 to 50%.

This same pattern did not extend to R. solani disease severity. Again, low disease susceptibility was considered to be a mean disease severity not significantly different from the genotype exhibiting the lowest overall disease severity within the R. solani screens. The percentages of genotypes within each PI/cultivar that exhibited low R. solani disease severity ranged from 0 to 35%. PI/cultivars with the highest percentage of genotypes exhibiting low R. solani disease severity were Falcon II (35%), Rebel (35%), Millennium (30%), Asheville (25%), and PI 561430 (Kentucky 31 E-) (21%). The remaining PIs had percentages <20%.

Forty genotypes with significantly lower R. solani disease severity, 122 genotypes with significantly lower R. zeae disease severity, and 26 genotypes with significantly lower disease severity to both pathogens were identified. These genotypes are being used as a source for developing cultivars with improved resistance to both pathogens.

Discussion

Brown patch is a highly destructive disease on tall fescue. Although cultivars are being developed with resistance to R. solani, the causal agent of brown patch, cultivar rankings for brown patch disease severity vary widely by location. Many factors probably influence that variability, including between- and within-location differences in inoculum loads and timing, isolate virulence, environmental conditions, and cultural practices. Based on results from this study, an additional contributing factor may be the influence of R. zeae, a pathogen that causes symptoms indistinguishable from those of R. solani in the field.

Cultivars ranked moderate to high for brown patch resistance in the NTEP did not exhibit significantly higher R. solani resistance but did exhibit significantly higher R. zeae resistance when compared with the remaining PIs in the study. A significant interaction was observed between genotype and pathogen, and correlations between R. solani and R. zeae disease severity were weak and not significant. These results suggest that resistance to R. solani does not equate to resistance to R. zeae; however, the interaction between PI and pathogen was not significant. This finding may reflect the large amount of variation in disease resistance among genotypes within each PI. Tall fescue is a self-incompatible allohexapoloid (Seal 1983). This results in genetically diverse populations, both in landraces and in developed cultivars, which are often synthetics or improved heterogenous populations (Pedersen and Sleper 1993). Further examination of a larger population of both landraces and cultivars bred for brown patch resistance, as well as inclusion of more cultivars on which previous NTEP brown patch ratings are available, could provide further insight into differences in PI × pathogen interaction.

Average disease severity was lower for *R. zeae* (36.3%) than for *R*. solani (48.6%). This finding is contrary to results found by Martin and Lucas (1984) in which R. zeae isolates tested in greenhouse conditions were as virulent as R. solani isolates. Although results from other studies have identified that virulence can vary by isolate, no significant interaction between isolate and genotype has been found when screening other turfgrass species-pathogen combinations, including dollar spot (Sclerotinia homoeocarpa F.T. Bennett) on seashore paspalum (Paspalum vaginatum Swartz) (Stekettee et al. 2016) and gray snow mold (Typhula incarnata Lasch) on bentgrass species (Agrostis stolonifera L., Agrostis capillaris L., Agrostis canina L.) (Chang et al. 2007). Given that three isolates per species were used, all of which were shown through preliminary studies to be highly virulent on tall fescue, genotype × isolate interaction is unlikely. However, total disease severity by species could differ under a different subset of isolates.

In the transition zone, R. zeae has been isolated at lower frequencies compared with R. solani and exhibits regional variation in prevalence (Amaradasa et al. 2017; Koehler and Shew 2017). This variation may contribute to the regional differences in rankings of brown patch-resistant cultivars when improved cultivars have been initially screened under controlled conditions using only R. solani or under field conditions where R. solani is dominant. Breeding for resistance to R. solani and R. zeae could result in varieties that exhibit fewer brown patch symptoms across a broader geographic footprint. Genotypes identified in this study will be used to develop cultivars with improved resistance to both pathogens.

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