***Brachypodium*: A Potential Model Host for Fungal Pathogens of Turfgrasses**

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ABSTRACT

Brachypodium distachyon is a C3 grass that is an attractive model host system for studying pathogenicity of major turfgrass pathogens due to its genetic similarity to many cool-season turfgrasses. Infection assays with two or more isolates of the casual agents of dollar spot, brown patch, and Microdochium patch resulted in compatible interactions with *B. distachyon* inbred line Bd21-3. The symptoms produced by these pathogens on Bd21-3 closely resembled those observed on the natural turfgrass host (creeping bentgrass), demonstrating that *B. distachyon* is susceptible to the fungal pathogens that cause dollar spot, brown patch, and Microdochium patch on

turfgrasses. The interaction between *Sclerotinia homoeocarpa* isolates and *Brachypodium* ecotypes was also investigated. Interestingly, differential responses of these ecotypes to *S. homoeocarpa* isolates was found, particularly when comparing *B. distachyon* to *B. hybridum* ecotypes. Taken together, these findings demonstrate that *B. distachyon* can be used as a model host system for these turfgrass diseases and leveraged for studies of molecular mechanisms contributing to host resistance.

Additional keywords: host–pathogen interactions, snow mold.

Turfgrass is the largest irrigated crop in the United States at roughly 35 million acres of land (Breuninger et al. 2013; Leinauer and Devitt 2013). Additionally, turf provides many functional, recreational, and ornamental benefits to human activities, making it an important provider of ecosystem services (Beard 1973). Professional turfgrass sectors include golf courses, sod production, institutional facilities, and management services such as lawn care and irrigation. Regardless of the turf sectors, high-quality turf is essential and is generally achieved through repeated applications of pesticides for weed, insect, and disease control. It is estimated that fungicide applications for disease management cost the golf industry alone \$174.40 million per year in the United States (Kerns and Tredway 2013). A single golf course may spend \$20,000 to 50,000 a year on fungicide applications for putting greens, a small but intensively managed part of the golf course. In addition to the economic costs associated with repeated fungicide applications, nontarget effects of fungicides can be hazardous to the environment (Baird et al. 1999; Baris et al. 2010) and the development of fungicide resistance in target pathogens is a common result of repeated exposure to a single active ingredient (Latin 2011). Biological and cultural controls offer alternatives to frequent fungicide applications but fail to consistently achieve adequate disease suppression comparable with that of chemical management tactics (Koppenhöfer et al. 2013).

Breeding popular turfgrass species for better disease resistance is the primary goal for sustainable disease management in golf course settings (Bonos et al. 2006). Due to the lack of genomic sequence data and other genetic tools for major turfgrass species, breeding for disease resistance has largely been based on traditional methods and has proceeded at a slow, albeit steady, pace (Belanger et al. 2004; Bonos et al. 2004, 2006; Studer et al. 2007). A biologically relevant model host for important turfgrass pathogens would enable precise identification of key genes involved in disease resistance or susceptibility. These genes could then be specifically targeted in turfgrass breeding efforts to produce a more streamlined approach toward development of disease-resistant cultivars (Spangenberg

et al. 2005). Unfortunately, an ideal model host for turfgrass pathogenesis studies has not previously been identified.

The genus *Brachypodium* includes C3 grass species in the subfamily Pooideae and is closely related to the major cool-season grass tribes Avenae, Bromae, Poeae, and Triticeae (Kellogg 2001). Just over a decade ago, a member of this genus, *Brachypodium distachyon* (L.) P. Beauv., was proposed as a novel model system for both turf-type or forage grasses and temperate cereals (Draper et al. 2001). More recently, the biofuels industry has adopted *B. distachyon* as a model due to its similarities with key bioenergy crops, including *Miscanthus* spp. and switchgrass (Gomez et al. 2008; Zhao et al. 2011). Much like the dicot model plant *Arabidopsis thaliana*, *B. distachyon* is small, fast growing, and amenable to inbreeding and has a simple diploid genome (Draper et al. 2001). Genetic resources for *B. distachyon* have steadily increased since its emergence as a model species, including the release of a high-quality, annotated complete genomic sequence in 2010 (The International Brachypodium Initiative 2010). The availability of a genomic sequence and accompanying genetic resources (Bragg et al. 2012; Garvin et al. 2008) (<http://brachybase.org>; <http://www.phytozome.net>; <http://www.brachypodium.org>) have encouraged widespread use of *B. distachyon* as a model for surveys of evolutionary genetics in grasses and comparisons of grass genomes (Bossolini et al. 2007; The International Brachypodium Initiative 2010). *B. distachyon* has also been successfully used in multiple cases to generate and refine linkage maps for cool-season grasses (Chutimanitsakun et al. 2011; Zhang et al. 2010; Zhao et al. 2011) and is considered the optimal model plant for members of the *Poaceae* family (Lyons and Scholthof 2015).

Host–pathogen interactions between *B. distachyon* and plant pathogens have previously been described for a number of economically important monocot diseases (Ayliffe et al. 2013; Barbieri et al. 2011; Figueroa et al. 2013; Parker et al. 2008; Peraldi et al. 2011, 2014; Routledge et al. 2004; Sandoya and de Oliveira Buanafina 2014; Schneebeli et al. 2016; Zhong et al. 2015) and a review on the status of *Brachypodium* as an emerging model for cereal pathosystems was recently provided by Fitzgerald and colleagues (2015). The earliest of these studies focused on the interaction between *B. distachyon* and the model pathogen *Magnaporthe grisea*, causal agent of blast disease on rice and other grasses (Routledge et al. 2004). Not only did this research demonstrate the utility of *B. distachyon* as a model for host–pathogen interactions but it also

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demonstrated that the *B. distachyon*–*M. grisea* interaction mirrored interactions of this pathogen with rice at both the histological and molecular levels, including the presence of resistance conferred by a single dominant gene (Routledge et al. 2004).

B. distachyon has also been proposed as an optimal model host for studying Fusarium diseases of wheat because infection of *B. distachyon* is remarkably similar to the infection process observed in wheat, including infection of *B. distachyon* spikes by the Fusarium head blight pathogens *Fusarium graminearum* and *F. culmorum* (Peraldi et al. 2011). Compatible interactions have also been described between *B. distachyon* and *Oculimacula* spp. and *B. distachyon* and *Ramularia collo-cygni*, the causal agents of eyespot and Ramularia leaf spot of cereal, respectively (Peraldi et al. 2014). Multiple researchers have characterized the interactions between *Brachypodium* spp. and various cereal rust pathogens, including *Puccinia graminis*, *P. brachypodii*, *P. triticea*, and *P. striiformis* (Ayliffe et al. 2013; Barbieri et al. 2011; Figueroa et al. 2013). Collectively, these studies have demonstrated a range of responses to rust pathogens in different *Brachypodium* ecotypes and the utility of *Brachypodium* spp. as a model system for studying molecular components of incompatibility and nonhost resistance to rust pathogens.

The present research aims to extend the body of work on *Brachypodium* spp.–cereal pathogen interactions by evaluating the compatibility of *B. distachyon* with three major fungal pathogens of turfgrass: *Sclerotinia homoeocarpa*, *Microdochium nivale*, and *Rhizoctonia solani*. In addition, this research explores the genetic diversity present in wild ecotypes in response to *S. homoeocarpa* isolates and paves the way for widespread use of *Brachypodium* spp. as a model for turfgrass pathogens.

MATERIALS AND METHODS

Biological materials. All pathogen isolates (Table 1) were obtained from symptomatic turfgrass using standard methods or were obtained from colleagues. Briefly, turfgrass blades displaying symptoms characteristic for dollar spot, Microdochium patch, or

brown patch were surface disinfested in changed 10% Clorox solution and rinsed with distilled water. Individual blades were then excised and placed on antibiotic- or pH-amended media and incubated for at least 48 h. Hyphal tips of colonies growing from symptomatic leaf areas and resembling the pathogens of interest in growth rate, growth pattern, and mycelial pigmentation were excised and transferred to fresh media to obtain pure cultures. Cultures were identified as the pathogens of interest based on colony morphology, conidial production and morphology, and sequencing of the internal transcribed spacer (ITS) region using conserved fungal (ITS 4 and 5) primers (White et al. 1990). Cultures were maintained in the dark at $21 \pm 2^\circ\text{C}$ on potato dextrose agar (PDA) plates and transferred weekly to fresh media. Additionally, long-term stocks of all isolates were stored at -80°C on filter paper disks.

Seed for *B. distachyon* inbred line Bd21-3 was obtained from researchers at Cornell University. *B. distachyon* accessions (Table 2) were ordered from the United States Department of Agriculture National Plant Germplasm System (<http://www.ars-grin.gov/npgs>) and have previously been assessed for leaf and stripe rust susceptibility (Barbieri et al. 2011). *B. distachyon* was cultivated in 8-cm-diameter pots filled with a 50:50 (vol/vol) mixture of calcine clay (Turface, Buffalo Grove, IL) and potting mix. Pots were placed into plastic flats to allow for bottom watering and a single seed was placed in each pot. To promote germination, a propagation dome was placed over pots for the first 3 to 5 days after sowing. This was then removed to allow for plant growth. Adequate plant nutrition was maintained by bottom-watering once weekly with quarter-strength Miracle-Gro Water Soluble All-Purpose Plant Food (The Scotts Company, LLC, Marysville, OH). For the first 3 weeks of growth, all plants were maintained in a growth room with a 14-h photoperiod at $24 \pm 2^\circ\text{C}$, with a fluorescent light intensity of 225 to 275 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Plants to be inoculated with *R. solani*, *S. homoeocarpa*, and *M. nivale* were moved to the appropriate controlled-environment chambers 4 weeks after seeding to allow time for acclimation to inoculation conditions prior to pathogen challenge.

TABLE 1. Fungal isolates used for *Brachypodium distachyon* inoculations

Species	Isolate code	Collection site	Host species	Disease
<i>Rhizoctonia solani</i> (AG2-2 IIIB)	Rs	North Carolina	Creeping bentgrass	Brown patch
<i>R. solani</i> (AG2-2 IIIB)	SRSE	North Carolina	Creeping bentgrass	Brown patch
<i>Microdochium nivale</i>	BH7	Wisconsin	Creeping bentgrass	Snow mold
<i>M. nivale</i>	MN5	Illinois	Creeping bentgrass	Snow mold
<i>Sclerotinia homoeocarpa</i>	2F92-1 ^y	Wisconsin	Creeping bentgrass	Dollar spot
<i>S. homoeocarpa</i>	Heath1A	Wisconsin	Creeping bentgrass	Dollar spot
<i>S. homoeocarpa</i>	LFD8	North Carolina	Bermudagrass	Dollar spot
<i>S. homoeocarpa</i>	OJN9 ^z	Wisconsin	Creeping bentgrass	Dollar spot
<i>S. homoeocarpa</i>	S10 ^z	Wisconsin	Creeping bentgrass	Dollar spot

^y Previously described by Koch et al. (2009).

^z Previously described by Rioux et al. (2014).

TABLE 2. *Brachypodium* accessions used for fungal inoculations and accession–isolate interaction evaluations

Species ^z	Accession ID	Ploidy level	Origin	Inbred lines
<i>Brachypodium distachyon</i>	Bd21-3	Diploid	Iraq	...
<i>B. distachyon</i>	PI 245730	Diploid	Turkey	Bd18-1
<i>B. distachyon</i>	PI 254867	Diploid	Iraq	Bd21, Bd21-3
<i>B. distachyon</i>	PI 639818	Diploid	Ukraine	Bd29-1
<i>B. hybridum</i>	PI 226629	Allotetraploid	Iran	Bd11-1, Bd11-2
<i>B. hybridum</i>	PI 227011	Allotetraploid	Iran	Bd12-1, Bd12-2
<i>B. hybridum</i>	PI 233228	Allotetraploid	Israel	Bd13-1, Bd13-2
<i>B. hybridum</i>	PI 239713	Allotetraploid	Iran	Bd14-1, Bd14-2
<i>B. hybridum</i>	PI 239714	Allotetraploid	Iran	Bd15-1, Bd15-2
<i>B. hybridum</i>	PI 254868	Allotetraploid	Iraq	Bd22-1, Bd22-2
<i>B. hybridum</i>	PI 287783	Allotetraploid	Spain	Bd23-1, Bd23-2
<i>B. hybridum</i>	PI 372187	Allotetraploid	Uruguay	Bd26-1, Bd26-2
<i>B. hybridum</i>	PI 533105	Allotetraploid	Australia	Bd28

^z Species designations are based on the reclassification of *Brachypodium* spp. based on ploidy level and other characteristics by Catalan and colleagues (2012).

Bd21-3 growth chamber infection assays. Infection assays of Bd21-3 with all three fungal pathogens were set up as a randomized complete block design (RCBD) with three replicates, and each study was repeated three times. The majority of inoculations were performed using the Parafilm sachet method (Jia et al. 2012). Briefly, a 6-mm-diameter agar plug was excised from the advancing edge of fungal colonies with a sterile cork borer and adhered to the plant, mycelium-side against the plant, with a thin strip of Parafilm. Plugs were applied 2 to 4 cm from the base of the plant. Control plants were mock inoculated with PDA plugs lacking mycelium. Due to differing growth rates of the fungal pathogens used in these studies, the age of cultures when used for inoculation varied, as described below.

S. homoeocarpa inoculations were performed in a controlled-environment room held at $24 \pm 2^\circ\text{C}$ with a 14-h photoperiod. Five-day-old cultures were used for inoculation. The Parafilm sachet method alone was adequate for inducing consistent symptom development. Disease severity, assessed as the percentage of tissue affected, was rated every 24 h using the Horsfall-Barratt scale (Horsfall and Barratt 1945). To determine pathogenicity of *S. homoeocarpa* on *B. distachyon*, an initial assay was conducted with Bd21-3 and four Wisconsin isolates originally collected from creeping bentgrass (2F92-1, Heath1A, OJN9, and S10). Following successful demonstration of infection on Bd21-3, two of these isolates (2F92-1 and OJN9) and LFD8, a North Carolina isolate from bermudagrass, were used for screening against *Brachypodium* ecotypes. LFD8 was included to increase the geographic and host diversity of isolates used in this study.

A controlled-environment chamber at $28 \pm 4^\circ\text{C}$ with a 10-h photoperiod was used for *R. solani* inoculations. *R. solani* cultures were 5 days old at the time of inoculation. To achieve consistent

disease pressure and symptom development, relative humidity was maintained at or near 100% by covering flats with humidity domes. Disease severity was rated at 5 days postinoculation, as described for *S. homoeocarpa* above. The experiment was conducted as an RCBD with four replicates for each treatment within each of three experimental repetitions, for a total of 12 data points per isolate.

Due to the slower growth of *M. nivale*, inoculations were performed using 7-day-old cultures. Inoculated plants were placed in a growth chamber held at a constant temperature of 21°C with a 14-h photoperiod. The lower temperature mimicked the cool conditions under which *M. nivale* typically infects. A humidity dome was placed over inoculated plants to maintain high relative humidity and disease severity ratings were performed at 5 days postinoculation, as described above. Similar to *R. solani*, there were four treatment replicates within each of three experimental repetitions.

Following the 5-day disease incubation period and rating of symptom severity, all plants were photographed to document and compare symptoms. Pathogens were reisolated from symptomatic material to confirm presence of the inoculated pathogen. This was done by briefly surface disinfecting diseased *B. distachyon* foliage in 10% Clorox solution, rinsing with sterile deionized water, and plating small pieces of the diseased plant material on antibiotic-amended medium. Plates were incubated at room temperature for up to 1 week to allow time for the target fungi to grow, and identity was confirmed by colony morphology.

***Brachypodium* natural ecotype by *S. homoeocarpa* isolate infection assays.** Twelve natural ecotypes, as well as the inbred line Bd21-3, and three *S. homoeocarpa* isolates were used for ecotype-isolate interaction infection assays. *Brachypodium* ecotypes differed in ploidy level and included representatives from

TABLE 3. Specific comparisons of symptom severity for *Brachypodium* ecotype–*Sclerotinia homoeocarpa* isolate interactions^w

Isolates ^x	Group 1 ^y	Group 2 ^z	Group 1 mean (SE)	Group 2 mean (SE)	F value	P value
All	<i>B. distachyon</i> eco	<i>B. hybridum</i>	39.54 (2.88)	50.76 (1.80)	15.91	<0.0001***
	All <i>B. distachyon</i>	<i>B. hybridum</i>	42.55 (2.64)	50.76 (1.80)	12.61	0.0004***
	Bd21-3	PI 254867	48.33 (5.16)	43.70 (2.57)	0.69	0.4085
	Bd21-3	PI 245370	48.33 (5.16)	35.37 (4.04)	5.37	0.0215*
	Bd21-3	PI 639818	48.33 (5.16)	42.78 (4.75)	0.99	0.3214
	PI254867	PI 245730	43.70 (2.57)	35.37 (4.04)	2.36	0.1257
	PI 254867	PI 639818	43.70 (2.57)	42.78 (4.75)	0.03	0.8646
	PI 639818	PI 245730	42.78 (4.75)	35.37 (4.04)	1.86	0.1733
2F92-1	<i>B. distachyon</i> eco	<i>B. hybridum</i>	37.04 (4.27)	50.36 (3.11)	8.10	0.0054**
	All <i>B. distachyon</i>	<i>B. hybridum</i>	37.93 (3.15)	50.36 (3.11)	8.45	0.0045**
	Bd21-3	PI 254867	40.63 (9.23)	43.33 (6.45)	0.07	0.7897
	Bd21-3	PI 245370	40.63 (9.23)	28.89 (7.4)	1.34	0.2492
	Bd21-3	PI 639818	40.63 (9.23)	38.89 (5.58)	0.03	0.8642
	PI254867	PI 245730	43.33 (6.45)	28.89 (7.4)	2.16	0.1447
	PI 254867	PI 639818	43.33 (6.45)	38.89 (5.58)	0.20	0.6519
	PI 639818	PI 245730	38.89 (5.58)	28.89 (7.4)	1.86	0.1733
OJN9	<i>B. distachyon</i> eco	<i>B. hybridum</i>	44.81 (3.83)	57.24 (2.73)	7.44	0.076**
	All <i>B. distachyon</i>	<i>B. hybridum</i>	48.14 (4.29)	57.24 (2.73)	4.88	0.0296*
	Bd21-3	PI 254867	58.13 (5.08)	39.44 (5.86)	3.40	0.0682
	Bd21-3	PI 245370	58.13 (5.08)	42.78 (6.88)	2.30	0.1330
	Bd21-3	PI 639818	58.13 (5.08)	52.22 (8.04)	0.34	0.5614
	PI254867	PI 245730	39.44 (5.86)	42.78 (6.88)	1.12	0.1352
	PI 254867	PI 639818	39.44 (5.86)	52.22 (8.04)	1.69	0.1966
	PI 639818	PI 245730	52.22 (8.04)	42.78 (6.88)	0.92	0.3389
LFD8	<i>B. distachyon</i> eco	<i>B. hybridum</i>	40.00 (4.24)	44.70 (2.17)	1.44	0.2323
	All <i>B. distachyon</i>	<i>B. hybridum</i>	41.56 (3.38)	44.70 (2.17)	0.78	0.3800
	Bd21-3	PI 254867	46.25 (7.06)	48.33 (5.65)	0.06	0.8073
	Bd21-3	PI 245370	46.25 (7.06)	34.44 (5.56)	1.92	0.1691
	Bd21-3	PI 639818	46.25 (7.06)	37.22 (5.72)	1.12	0.2920
	PI254867	PI 245730	48.33 (5.65)	34.44 (5.56)	2.82	0.0961
	PI 254867	PI 639818	48.33 (5.65)	37.22 (5.72)	1.81	0.1820
	PI 639818	PI 245730	37.22 (5.72)	34.44 (5.56)	0.11	0.7357

^w SE = standard error.

^x *S. homoeocarpa* isolates.

^y “*B. distachyon* eco” (ecotypes) includes the three *Brachypodium distachyon* wild-type accessions but not Bd21-3 and “All *B. distachyon*” includes the three *B. distachyon* wild-type accessions and Bd21-3.

^z “*B. hybridum*” indicates all *B. hybridum* accessions.

the species *B. distachyon* and *B. hybridum* (Table 3). Four-week old plants were used for infection assays and plants were maintained as previously described. Infection assays were performed in a growth room held at $24 \pm 2^\circ\text{C}$ with a 14 h day-length. The experiment was designed as a factorial within an RCBD of four treatments (three *S. homoeocarpa* isolates and PDA control) by 13 *Brachypodium* accessions, with three replications. Treatments and accessions were randomly assigned within each block and the entire experiment was repeated three times. Blocks were treated as nested within experimental repetitions for statistical analysis. Inoculations were carried out as previously described for *S. homoeocarpa*. At 5 days postinoculation, disease severity on individual plants was rated with the Horsfall-Barratt scale and plants were photographed to allow for comparison of infection type and symptom severity.

Statistical analysis. All data were analyzed using SAS software v.9.3 (SAS Institute, Cary, NC). Horsfall-Barratt ratings were converted to the geometric mean of the percentage range associated with each rating index, which resulted in normal distribution of the data as judged by residual plots and tests for normality within PROC UNIVARIATE. Bd21-3 infection assays

with *S. homoeocarpa*, *R. solani*, and *M. nivale* were analyzed using the PROC MIXED procedure. For analysis of Bd21-3 data, treatment was considered a fixed main effect and experimental replicate was considered a random blocking factor. Analysis of variance (ANOVA) was used to compare treatment means and single degree-of-freedom contrast statements were used to make preplanned comparisons between isolates and inoculated versus mock-inoculated controls. Studentized residual plots and model-fitting criteria (Akaike Information Criterion) were generated and assessed to confirm appropriateness of the models used. When not significant, random factors and interactions were removed from the model used for final analysis if the model-fitting criteria indicated a better fit in the absence of these terms.

S. homoeocarpa isolate $\times$ *B. distachyon* accession data were analyzed with the PROC GLIMMIX procedure to allow for the use of slicing to compare levels of one main factor within the different levels of the other. Isolate and accession were both treated as fixed main effects. Initially, block and block(repetition) were treated as random effects. Block(repetition) did not contribute significantly to experimental variability and was excluded from the final analysis. ANOVA was used to compare means for main effects and single degree-of-freedom orthogonal contrast statements were used to make preplanned means comparisons between specific treatments. The SLICE option was used to allow for orthogonal contrast statements comparing levels of one factor within those of the other.

RESULTS

***S. homoeocarpa* symptoms, disease severity, and time-course of infection on Bd21-3.** All *S. homoeocarpa* isolates tested were pathogenic on Bd21-3 (Fig. 1). A small amount of bleaching occurred around the site of inoculation in the control pots that received mock inoculation with PDA. This resulted in the approximately 5% disease severity observed for control plants and was likely due to moderate phytotoxicity of PDA or facilitation of growth by saprophytic fungi naturally present on the grass. Symptoms were similar to those observed on creeping bentgrass in the lab and other turfgrass species under field conditions, including tan lesions with reddish-brown borders (Fig. 2A) (Walsh et al. 1999). Occasionally, hourglass-shaped lesions, a hallmark symptom of *S. homoeocarpa* infection, were observed on stems, sheaths, and leaves (Fig. 2B). In addition to characteristic lesions, necrotic flecking, possibly indicative of a hypersensitive response, was frequently observed in the early stages of infection (24 to 48 h postinoculation [hpi]) (Fig. 2C). Chlorosis, often leading to necrosis, occasionally presented along leaf margins (Fig. 2D). At 5 days following infection, individual leaf blades appeared wilted and necrotic (Fig. 2E). In severe infections, the entire plant became necrotic and wilted (Fig. 2F).

***Brachypodium* ecotype-*S. homoeocarpa* isolate interactions.** To determine the presence of natural resistance to *S. homoeocarpa* in *Brachypodium* spp., thirteen *Brachypodium* ecotypes were inoculated with three isolates of *S. homoeocarpa* collected from both C3 and C4 turfgrass hosts and symptom severity was compared (Table 4). Two of the isolates employed in these experiments were selected from the four Wisconsin isolates initially used to determine pathogenicity on Bd21-3. An additional isolate, LFD8, collected from bermudagrass in North Carolina, was included to increase the host and geographic diversity of *S. homoeocarpa* isolates tested. Due to the separation of polyploid ($2n = 30$) *B. distachyon* accessions into the new species *B. hybridum*, this study included three *B. distachyon* ecotypes, nine *B. hybridum* ecotypes, and one inbred *B. distachyon* line (Bd21-3).

Both accession and isolate had strong effects on symptom severity ($P = 0.0002$ for both) but an accession-isolate interaction was not detected ($P = 0.4965$), indicating that different *S. homoeocarpa* isolates produced similar symptom severity across the *Brachypodium* ecotypes assessed in this study. Orthogonal contrasts revealed that

Fig. 1. Disease severity of *Sclerotinia homoeocarpa* isolates on Bd21-3 at 5 days postinoculation (dpi). **A**, Average disease severity rating of whole *Brachypodium distachyon* plants 5 dpi with one of four *S. homoeocarpa* isolates or a potato dextrose agar (PDA) plug. Disease severity ratings represent the mean severity from three experimental repetitions with three plants per experiment ($n = 9$). Error bars represent $\pm$ one standard error of the mean. **B**, Progression of infection on Bd21-3 by four isolates of *S. homoeocarpa* and the mock-inoculated PDA control. Symptom severity was scored visually every 24 h for six days. Each data point represents the mean severity from three experimental repetitions with three plants per experiment ($n = 9$). Error bars represent $\pm$ one standard error of the mean.

disease severity was greater in *B. hybridum* ecotypes than *B. distachyon* ecotypes ($P < 0.0001$) (Table 3). Bd21-3 is an inbred line developed from the *B. distachyon* wild-type accession PI 254867; consequently, the response of these accessions to *S. homoeocarpa* was of interest but no difference was found between these two lines ($P = 0.3553$). To test for differences in resistance among individual *B. distachyon* accessions to *S. homoeocarpa*, single degree-of-freedom orthogonal contrasts were used to compare the two *B. distachyon* ecotypes PI 245730 and PI 639818 to the inbred line Bd21-3 (Table 3). Disease severity was similar between PI 639818 and Bd21-3 but a moderate difference in severity was detected between PI 245730 and Bd21-3 ($P = 0.02$). Additional orthogonal contrasts between individual *B. distachyon* ecotypes were performed but no further differences in susceptibility to *S. homoeocarpa* were detected (Table 3).

The majority of *Brachypodium* ecotypes do not differ in their relative resistance to *S. homoeocarpa* (Table 4). PI 245730 and PI 639818 were more resistant to *S. homoeocarpa* isolates than PI 227011. PI245730 was also more resistant than PI239713; otherwise, resistance to *S. homoeocarpa* did not differ between ecotypes. Because isolate was significant in the overall analysis, means separations were also performed within individual isolates (Table 4). Similar to the overall test, PI227011 and PI239713 had the highest disease severity for both C3 *S. homoeocarpa* isolates in this study (Table 4). PI245730 had significantly lower symptom severity than the two least resistant ecotypes for both C3 *S. homoeocarpa* isolates. PI227011 and PI239713 were not significantly different from PI245730 in symptom severity when inoculated with the C4 *S. homoeocarpa* isolate (Table 4). PI245730 again had the lowest symptom severity,

along with PI 226629. Only PI 287783 had greater symptom severity than these two ecotypes (Table 4).

***R. solani* symptom development and severity on Bd21-3.** Both *R. solani* isolates used in this experiment were pathogenic on Bd21-3 and there was no difference in aggressiveness between the two isolates (Fig. 3A). Symptoms produced on Bd21-3 were similar to those produced on creeping bentgrass under artificial conditions (Chai et al. 2002) and to those reported in the field on many turfgrass species (Smiley et al. 2005). Plant symptoms included tan to gray lesions along leaf blades with dark-brown borders. Chlorosis spreading along leaf blades frequently accompanied these lesions (Fig. 3B and C). Lesion phenotype varied from small and circular to long and irregularly shaped (Fig. 3B and C). Tips of severely infected leaf blades occasionally became necrotic and tissue surrounding the site of inoculation was frequently damaged (Fig. 3C). Symptoms were primarily observed on leaf blades and rarely advanced to stems and sheaths. Sparse white mycelia were occasionally produced in infected areas of host tissue.

***M. nivale* symptom development and severity on Bd21-3.** *M. nivale* isolates used in this study were pathogenic on Bd21-3 but differed significantly in aggressiveness (Fig. 4A). Symptoms produced by BH7 generally included necrosis of leaf blades that occasionally spread to ligules, sheaths, and culms. Chlorosis was observed spreading along infected leaf blades (Fig. 4B). Sparse white mycelia were frequently observed on infected plants (Fig. 4B). The more aggressive isolate, MN5, produced large, dark-brown necrotic lesions affecting blades, ligules, sheaths, and culms (Fig. 4C). Chlorosis was frequently noted in leaf blades and spread beyond areas of necrosis (Fig. 4D). Severely affected leaf blades were completely necrotic and occasionally appeared wilted (Fig. 4C). As with isolate BH7, sparse white mycelia were formed in areas of necrotic tissue (Fig. 4C).

DISCUSSION

The aim of the present study was to determine the potential for use of *B. distachyon* as a model host for three major fungal pathogens of amenity turfgrasses. Additionally, this research investigated pathogenesis and ecotype-isolate interactions of various *Brachypodium* accessions with the dollar spot pathogen *S. homoeocarpa*. The results of these studies clearly demonstrate compatible interactions between Bd21-3 and *S. homoeocarpa*, *M. nivale*, and *R. solani*. Additionally, screening of diverse *Brachypodium* ecotypes with C3 and C4 isolates of *S. homoeocarpa* indicated that genetic variability exists within *Brachypodium* accessions that can be leveraged for studies of host-pathogen interactions for *S. homoeocarpa* and other prominent fungal pathogens of turfgrass.

Fig. 2. Symptoms of *Sclerotinia homoeocarpa* on Bd21-3. **A**, Characteristic individual lesion, with white center and reddish brown border, frequently observed on infected foliage. **B**, Hallmark hourglass-shaped lesion on Bd21-3 stem and sheath. **C**, Necrotic flecking frequently observed early in infection and possibly indicative of a hypersensitive-type response to infection. **D**, Mild infection resulting in chlorosis of affected foliage and mild necrosis. **E**, Moderate infection of Bd21-3 by *S. homoeocarpa* at 5 days postinoculation resulting in necrosis and wilting of some leaf blades while others remain relatively unaffected. **F**, Widespread necrosis and wilting of leaf blades with spread of symptoms to sheaths and stems.

TABLE 4. Means separation for symptom severity of *Brachypodium* accessions inoculated with *Sclerotinia homoeocarpa*

Accession	Means separation ²			
	All isolates	2F92-1	OJN9	LFD8
PI 227011	62.4 A	70.0 A	68.9 A	48.3 AB
PI 239713	58.1 AB	59.4 AB	65.0 A	50.0 AB
PI 287783	51.2 ABC	47.8 BDC	52.8 ABC	52.9 A
PI 533015	51.0 ABC	45.0 BDC	58.1 ABC	50.0 AB
PI 254868	48.0 ABC	53.9 ABC	50.7 ABC	39.4 AB
Bd21-3	48.3 ABC	40.6 BDC	58.1 ABC	46.3 AB
PI 372187	49.2 ABC	45.6 BDC	63.1 AB	38.2 AB
PI 226629	46.9 ABC	47.5 BDC	60.0 AB	33.1 B
PI 239714	45.8 ABC	40.0 BDC	54.4 ABC	43.1 AB
PI 233228	44.3 BC	43.9 BDC	42.2 BC	46.7 AB
PI 254867	43.7 BC	43.3 BDC	39.4 C	48.3 AB
PI 639818	42.8 BC	38.9 DC	52.2 ABC	37.2 AB
PI 245730	35.4 C	28.9 D	42.8 BC	34.4 B

² Accessions with the same letter are not significantly different from each other by Fisher's protected least significant difference test, $\alpha = 0.05$.

Aside from dollar spot, brown patch is one of the most frequently encountered diseases on amenity turfgrass (Bokmeyer et al. 2009), and pink snow mold is the primary cool-season disease of turfgrasses throughout the globe (Smiley et al. 2005). In the present research, isolates of both *R. solani* and *M. nivale* caused symptoms on *B. distachyon* inbred line Bd21-3 under temperature and humidity conditions that are ideal for infection of turfgrass hosts by these pathogens. This suggests that both pathogens have requirements for infection on *B. distachyon* similar to those for natural turfgrass hosts. *R. solani* lesions on *B. distachyon* closely matched those observed previously on creeping bentgrass and other turfgrass species, including the formation of irregularly shaped tan lesions with dark-red to brown borders between diseased and healthy tissue (Smiley et al. 2005). Due to the lack of empirical data on development of brown patch symptoms on turfgrass in controlled settings, it is difficult to make additional direct comparisons; however, further studies of *R. solani* pathogenesis using *B. distachyon* as a model will contribute to better understanding of the physiological and molecular

mechanisms of turf-type grass infection by this pathogen. *R. solani* AG2-2 IIIB, the primary turfgrass-infecting anastomosis group (AG), affects other hosts in the *Poaceae* family (Ogoshi 1987). Information gained from the *B. distachyon* pathosystem can be extended to enhance understanding of *R. solani* pathogenesis in these hosts as well. The wheat root rot pathogen *R. solani* AG8 is also compatible with *B. distachyon*, and natural ecotypes vary in resistance to an isolate in this AG (Schneebeil et al. 2016). These results are consistent with the present research and indicate that *B. distachyon* may provide a general model pathosystem for *R. solani* AG that infect cool-season grasses.

In addition to being a major turfgrass pathogen, *M. nivale* is the causal agent of pink snow mold, also known as Microdochium patch, on winter wheat, rye, barley, and oat (Pettitt et al. 1993; Simpson et al. 2000). Some research on *M. nivale*-host interactions has been performed in the wheat pathosystem but the relatedness of *M. nivale* isolates from turf and cereal crop plants is not well understood (Jewell and Hsiang 2013; Lees et al 1995; Litschko and

Fig. 3. Infection of *Brachypodium distachyon* inbred line Bd21-3 with *Rhizoctonia solani*. **A**, Mean symptom severity of Bd21-3 5 days following inoculation with *R. solani* isolate Rs or SRSE or with a potato dextrose agar (PDA) plug. Single degree-of-freedom orthogonal contrast statements were used to compare between *R. solani* isolates and between *R. solani* isolates and the PDA control at $\alpha = 0.05$. NS = no significant difference detected and *** = $P < 0.0001$. **B**, Mild symptoms of *R. solani* infection on Bd21-3, including chlorosis and small, necrotic lesions. **C**, Moderate infection of Bd21-3 by *R. solani* with necrotic leaf tips, severe necrosis surrounding the site of inoculation, and the presence of sparse mycelia near the site inoculation. Error bars represent $\pm$ one standard error of the mean.

Fig. 4. Infection of *Brachypodium distachyon* inbred line Bd21-3 with *Microdochium nivale*. **A**, Mean symptom severity of Bd21-3 5 days following inoculation with *M. nivale* isolate BH7 or MN5 or with a potato dextrose agar (PDA) plug. Single degree-of-freedom orthogonal contrast statements were used to compare between *M. nivale* isolates and between *M. nivale* isolates and the PDA control at $\alpha = 0.05$; *** = $P < 0.0001$. **B**, Symptoms of less aggressive *M. nivale* isolate BH7 on Bd21-3. **C**, Moderate infection of Bd21-3 by *M. nivale* isolate MN5, including necrosis, wilting, and production of sparse mycelia at the site of infection. Error bars represent $\pm$ one standard error of the mean.

Burpee 1987; Simpson et al. 2000). Consequently, a model system for studying *M. nivale*–host interactions will be helpful for turfgrass pathology and may extend to additional economically important crops such as wheat, barley, and ryegrass. On individual turfgrass blades, symptoms of *M. nivale* infection are nondistinct, with generalized water soaking and necrosis, as well as the presence of tufts of aerial white mycelia (Smiley et al. 2005). The symptoms observed on *B. distachyon* plants artificially inoculated with agar plugs of *M. nivale* were remarkably similar (Fig. 4B and C). Dark, water-soaked lesions formed around the site of inoculation both upward into leaf blades and downward into culms. Similar to turfgrass infections, fluffy white mycelia were observed protruding from the border between healthy and diseased tissue. The name “pink snow mold” derives from the presence of pinkish mycelia and sporodochia that appear on infected turf, giving symptomatic areas a pinkish coloration. *M. nivale* sporulation is sunlight dependent; thus, this phenomenon was not observed on *B. distachyon* in the present study but may be possible with the right environmental conditions (Smiley et al. 2005). *M. nivale* reisolated from symptomatic *B. distachyon* plants produced abundant conidia in culture, indicating no negative effects on the fungus from exposure to *B. distachyon*.

Due to growing concerns over extensive fungicide use for dollar spot control and increasing issues with resistance to common fungicide groups in *S. homoeocarpa* populations (Burpee 1997; Miller et al. 2002; Orshinsky et al. 2012a, b; Putman et al. 2010), there has been a resurgence in research on *S. homoeocarpa* pathogenesis. Orshinsky and colleagues (2012a) used histological methods and microscopy to study the infection process of virulent, hypovirulent, and asymptomatic isolates of *S. homoeocarpa* on detached creeping bentgrass leaves. In these studies, plugs from 5-day-old PDA cultures of *S. homoeocarpa* were placed on leaf blades over moistened filter paper in Parafilm-sealed Petri plates. In agreement with our studies on intact plants, appressoria, particularly in association with stomata, and intercellular hyphal growth were observed by 48 hpi (Orshinsky et al. 2012a). In the current research, symptoms of *S. homoeocarpa* infection began appearing by 48 hpi and developed rapidly over the following days.

No *Brachypodium* accession–*S. homoeocarpa* isolate interaction was noted in this research, indicating that *S. homoeocarpa* employs a generalist pathogenesis strategy and that resistance–avirulence gene interactions do not occur between *S. homoeocarpa* and *B. distachyon*. This corresponds with interactions reported for turfgrass hosts and is part of the reason that breeding for dollar spot resistant turfgrass cultivars presents a significant challenge to turfgrass breeders (Bonos et al. 2003). It is likely that both natural hosts and *Brachypodium* spp. rely on quantitative resistance mechanisms to combat *S. homoeocarpa* infection. Identification of these mechanisms in *Brachypodium* spp. could be used to identify similar traits in turfgrasses.

Previous studies have demonstrated the utility of *B. distachyon* as a model host for various cereal rust pathogens that affect turfgrass species (Ayliffe et al. 2013; Barbieri et al. 2011; Beirn et al. 2011; Figueroa et al. 2013). The present research extends the applications of *B. distachyon* as a tool for studying host–pathogen interactions in turfgrasses to three additional major fungal diseases of these hosts: dollar spot, brown patch, and pink snow mold. No genomic sequence data is publicly available for popular turfgrass species, and large-scale transcriptomic analyses of endophyte or pathogen-infected turfgrass hosts are limited (Ambrose and Belanger 2012; Orshinsky et al. 2012b). Additionally, the only genetic tools currently available for genetic manipulation of turfgrass species is *Agrobacterium*-mediated transformation, which is time consuming and complicated by the complex genomes of most turfgrass species (Bajaj et al. 2006; Dong and Qu 2005; Ge et al. 2006; Luo et al. 2004). In contrast, the multitude of public genetic resources and molecular tools available for *B. distachyon*, as well as the ease with which this species can be cultivated, inbred, and inoculated, make it an ideal model for studying the molecular aspects of host resistance

to major turfgrass pathogens. In this research, *Brachypodium* accessions with significant differences in resistance to *S. homoeocarpa* were identified. Inbred lines derived from these accessions and selected for resistance or susceptibility to *S. homoeocarpa* will provide useful tools for identification of genes involved in host resistance to this pathogen. In the future, similar studies that evaluate the resistance of *Brachypodium* accessions to *R. solani* and *M. nivale* will be needed to identify the best accessions to use for dissection of host resistance mechanisms to these pathogens.

This research demonstrated that *B. distachyon* inbred line Bd21-3 is a compatible host to the causal agents of turfgrass dollar spot, brown patch, and pink snow mold diseases. These are three of the most economically important diseases of turfgrass and account for the vast majority of fungicide sprays in high-value turf settings. Symptoms of these diseases produced on Bd21-3 mimic those observed on natural turfgrass hosts and indicate that *B. distachyon* is a useful model pathosystem to study molecular host–pathogen interactions of these diseases. The use of *B. distachyon* as a model will accelerate the pace at which key genes underlying host resistance or susceptibility to these causal agents of these diseases are identified and will enhance breeding efforts for disease-resistant turfgrass cultivars. Targeted breeding efforts will decrease the time needed to produce turfgrass cultivars resistant to dollar spot, brown patch, and pink snow mold and will significantly decrease dependence upon chemical control, resulting in more sustainable and environmentally friendly turfgrass disease management.

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