Brachypodium: Exploration for use as a model host for fungal pathogens of turfgrasses

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Introduction

- Dollar spot, brown patch, and Microdochium patch are three of the most economically devastating fungal diseases of amenity turfgrasses worldwide.
- Mangement of these diseases is generally achieved through frequent fungicide applications.
- Disease resistant turfgrass cultivars are a viable management alternative but progress in this realm is hampered by limited understanding of how turfgrass pathogen interact with their hosts.
- *Brachypodium distachyon* is a C3 grass with an assortment of genetic resources and, due to its genetic similarity to many cool-season turfgrasses, is an attractive model system for studying host-pathogen interactions in major turfgrass pathosystems.
- The aims of this study were
  - i) to determine host status of the B. distachyon inbred line Bd 21-3 to three major fungal turfgrass pathogens and
  - ii) to evaluate natural variation of Brachypodium sp. ecotypes in response to *S. homoeocarpa* isolates.

Materials & Methods

Plant Materials

- Seed of *Brachypodium* sp. inbred lines and wild-type accessions were obtained from the USDA-GRIN, David Garvin (USDA-ARS), and William Kreuser (U. of Nebraska-Lincoln)
- Plants were cultivated in 8 cm diameter pots filled with a 50:50 (v:v) mixture of Turface and potting mix.
- Plant nutrition was maintained by bottom-watering once weekly with ¼-strength MiracleGro.
- For the first three weeks of growth, all plants were maintained in a growth room with a 14 h day-length at 24±2°C
- In the fourth week, plants were moved to growth chambers at inoculation temperatures, described below, to allow time for acclimation prior to pathogen challenge

Inoculum Preparation

- Pathogen isolates were originally collected from symptomatic turf
- Cultures were maintained on PDA at pathogen-appropriate temperatures
- Five to seven day-old cultures, depending on pathogen growth rate, were used for inoculations

Inoculation and Disease Rating

- All pathogens were inoculated onto *Brachypodium* plants using the parafilm sachet method
- Inoculated plants were incubated at pathogen-appropriate temperatures and disease severity was rated every 24h (Sh) or 5 dpi (Rs and Mn) using the Horsfall-Barratt scale

Statistical Analysis

- Horsfall-Barratt ratings were converted to the geometric mean of the percentage range associated with each rating index
- Analyses were performed using the PROC GLIMMIX procedure in SAS v. 9.3

Symptoms and histology of *Sclerotinia homoeocarpa* infection on Bd 21-3

Sclerotinia homoeocarpa infection assays were set-up in an RCBD with three replications and repeated three times. Cultures were grown at 24±2°C until they were five-days old. A 6mm diameter sterile cork borer was then used to excise agar plugs from the advancing colony edge. These were placed mycelium side down 2-4 cm from the base of the plant and wrapped with parafilm. Symptom severity was rated daily in disease severity experiments. In separate experiments, destructive samples were collected for microscopy. These samples were cleared with acetic acid:ethanol mixtures and stained with 0.01% trypan blue in lactophenol.

Figure 1. Symptoms of *S. homoeocarpa* on Bd 21-3. (A) A characteristic lesion with white center and reddish brown borders **(B)** Hallmark hourglass-shaped lesion on Bd 21-3 stem and sheath **(C)** Necrotic flecking frequently observed early in infection **(D)** Mild infection resulting in chlorosis **(E)** Moderate infection of Bd 21-3 by *S. homoeocarpa* at 5 dpi resulting in necrosis and wilting of some leaf blades **(F)** Widespread necrosis and wilting of leaf blades with spread of symptoms to sheaths and stems.

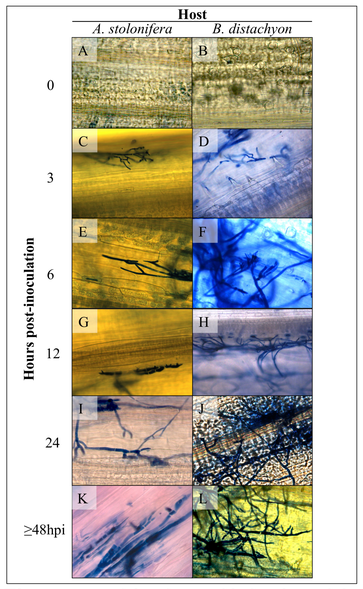

Figure 2. Time-course histology of infection of *A. stolonifera* and *B. distachyon* inbred line Bd 21-3 by *S. homoeocarpa*. (A, C, E, G, I, K) Progression of infection on the natural host *A. stolonifera* at 0, 3, 6, 12, 24, and ≥48 hpi, respectively. **(B, D, F, G, H, J, L)** Progression of infection on *B. distachyon* inbred line Bd 21-3 at 0, 3, 6, 12, 24, and ≥48 hpi, respectively.

Development of Rs infection on Bd 21-3

Rhizoctonia solani infection experiments were performed similarly to Sh infection experiments. To promote infection by Rs, which thrives at warmer temperatures than Sh, a growth chamber held at 28±4°C with a 10h day-length was used for Rs infection. Additionally, trays were covered with propagation domes to maintain humidity at or near 100%.

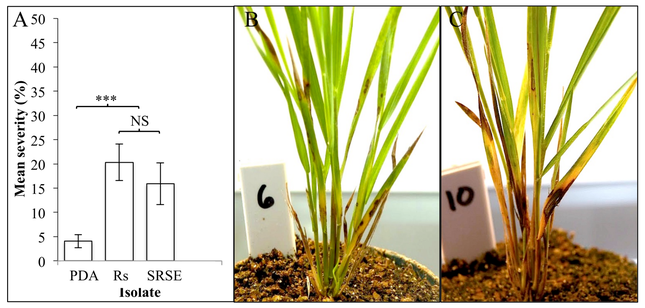

Figure 4. Infection of *B. distachyon* inbred line Bd 21-3 with *Rhizoctonia solani*. (A) Mean symptom severity of Bd 21-3 five days following inoculation with *R. solani* isolate Rs or SRSE, or with a PDA plug. Single degree-of-freedom orthogonal contrast statements were used to compare between *R. solani* isolates and the PDA control at α=0.05. NS = no significant difference detected and *** = $P < 0.0001$. **(B)** Mild symptoms of *R. solani* infection on Bd 21-3 including chlorosis and small, necrotic lesions. **(C)** Moderate infection of Bd 21-3 by *R. solani* with necrotic leaf tips, severe necrosis surrounding the site of inoculation, and the presence of sparse mycelia near the site inoculation.

Development of Mn infection on Bd 21-3

Experimental design and inoculation conditions for *Microdochium nivale* assays were similar to Sh and Rs. Due to the preference of Mn for cooler temperatures, cultures were grown for 7d at 21±2°C . Inoculations were performed in a 21±2°C growth chamber with a 14h day-length and plants were covered with a humidity dome to promote infection.

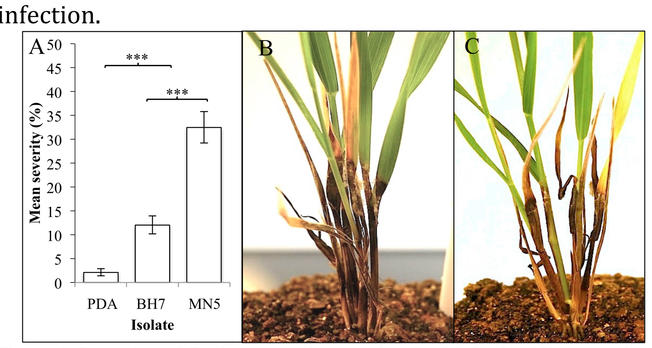

Figure 5. Infection of *B. distachyon* inbred line Bd 21-3 with *Microdochium nivale* (A) Mean symptom severity of Bd 21-3 five days following inoculation with *M. nivale* isolate BH7 or MN5, or with a PDA plug. Single degree-of-freedom orthogonal contrast statements were used to compare between *M. nivale* isolates and between M. Nivale isolates and the PDA control at α=0.05. *** = $P < 0.0001$. **(B)** Symptoms of less aggressive *M. nivale* isolate BH7 on Bd 21-3. **(C)** Moderate infection of Bd 21-3 by *M. nivale* isolate MN5, including necrosis, wilting, and production of sparse mycelia at the site of infection.

Interaction between *Sclerotinia homoeocarpa* isolates and *Brachypodium* sp. accessions

Infection experiments were performed similar to those described above except that additional *Brachypodium* sp. accessions were used (Table 1). The experiment was set up as a factorial (3 Sh isolates x 13 *Brachypodium* sp. accessions) within an RCBD with three replications and was repeated three times. Specific comparisons were made between agressiveness of Sh isolates collected from C3 versus C4 plants and symptom severity of diploid versus polyploid *Brachypodium* sp. accessions.

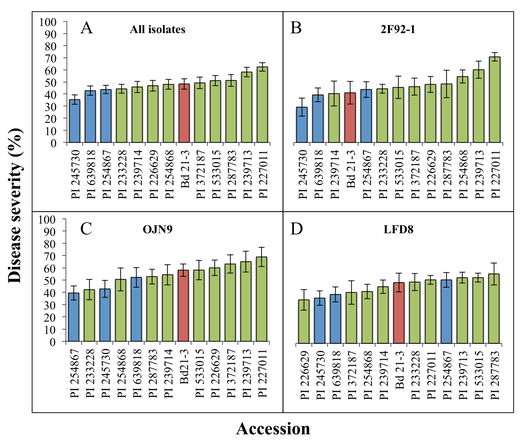

Figure 3. Symptom severity on *Brachypodium* species accessions five-days post-inoculation with *S. homoeocarpa*. (A) Mean symptom severity across all isolates tested. **(B)** Mean symptom severity for C3 isolate 2F92-1 **(C)** Mean symptom severity for C3 isolate OJN9. **(D)** Mean symptom severity for C4 isolate LFD8. Blue bars represent *B. distachyon* wild-type accessions; green bars represent *B. hybridum* wild-type accessions; and red bars represent *B. distachyon* inbred line Bd 21-3. Bars represent the mean of three replicate experiments with 2-3 plants per experiment (n=7-9) and errors bars represent ± one standard error of the mean.

Table 1. *Brachypodium* sp. accessions used in this research

Species	Accession ID	Ploidy Level	Origin	Inbred Lines
Brachypodium distachyon	Bd 21-3	Diploid	Iraq	-
Brachypodium distachyon	PI 245730	Diploid	Turkey	Bd18-1
Brachypodium distachyon	PI 254867	Diploid	Iraq	Bd21, Bd 21-3
Brachypodium distachyon	PI 639818	Diploid	Ukraine	Bd29-1
Brachypodium hybridum	PI 226629	Allotetraploid	Iran	Bd11-1, Bd11-2
Brachypodium hybridum	PI 227011	Allotetraploid	Iran	Bd12-1, Bd12-2
Brachypodium hybridum	PI 233228	Allotetraploid	Israel	Bd13-1, Bd13-2
Brachypodium hybridum	PI 239713	Allotetraploid	Iran	Bd14-1, Bd14-2
Brachypodium hybridum	PI 239714	Allotetraploid	Iran	Bd15-1, Bd15-2
Brachypodium hybridum	PI 254868	Allotetraploid	Iraq	Bd22-1, Bd22-2
Brachypodium hybridum	PI 287783	Allotetraploid	Spain	Bd23-1, Bd23-2
Brachypodium hybridum	PI 372187	Allotetraploid	Uruguay	Bd26-1, Bd26-2
Brachypodium hybridum	PI 533105	Allotetraploid	Australia	Bd28

Table 2. Specific comparisons of *Brachypodium* sp. accession x Sh isolate interactions

Sh Isolate(s)	Grp. 1	Grp. 2	Grp. 1 Mean (SE)	Grp. 2 Mean (SE)	F-value	P-value
All						
	C3	C4	50.46 (2.01)	43.73 (1.80)	8.57	0.004**
	Bd ecotypes[1]	Bh[2]	39.54 (2.88)	50.76 (1.80)	15.91	<0.0001***
	All Bd[3]	Bh	42.55 (2.64)	50.76 (1.80)	12.61	0.0004***
2F92-1						
	Bd ecotypes	Bh	37.04 (4.27)	50.36 (3.11)	8.10	0.0054**
	All Bd	Bh	37.93 (3.15)	50.36 (3.11)	8.45	0.0045**
OJN9						
	Bd ecotypes	Bh	44.81 (3.83)	57.24 (2.73)	7.44	0.076**
	All Bd	Bh	48.14 (4.29)	57.24 (2.73)	4.88	0.0296*
LFD8						
	Bd ecotypes	Bh	40.00 (4.24)	44.70 (2.17)	1.44	0.2323
	All Bd	Bh	41.56 (3.38)	44.70 (2.17)	0.78	0.3800

[1] 'Bd ecotypes' includes the three *B. distachyon* wild-type accessions but not Bd 21-3
[2] 'Bh' indicates all *B. hybridum* accessions
[3] 'All Bd' includes the three *B. distachyon* wild-type accessions and Bd 21-3

Conclusions

- *Brachypodium distachyon* inbred line Bd 21-3 is a suitable host for Sh, Rs, and Mn
- Disease symptoms on Bd 21-3 are similar to those on the natural host creeping bentgrass
- C3 and C4 isolates had different levels of aggressiveness on *Brachypodium* sp. accessions
- Symptom severity of C3 Sh isolates was greater on diploid *Brachypodium* sp. accessions than on polyploid *Brachypodium* sp. accessions, indicating that molecular mechanisms of resistance exist within these species