Characterization of the Response of Ornamental Graminoids to Attempted Infection by *Sclerotinia sclerotiorum*

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Abstract

Sclerotinia sclerotiorum causes Sclerotinia stem rot (SSR) (also called white mold), resulting in stem rot and death of many common herbaceous ornamental plant species. Resistant plants would be useful to manage SSR; however, the host range of *S. sclerotiorum* is unclear. The goal of this study was to determine how the ornamental graminoids *Pennisetum glaucum*, *Setaria italica*, *Juncus inflexus*, *Carex flagellifera*, *Isolepis cernua*, and *Acorus gramineus* respond to inoculation with *S. sclerotiorum*. Plants were inoculated in the field and in controlled environments at 13, 16, 19, or 22°C with or without wounding, and evaluated for SSR. Inoculated detached leaves were stained to examine infection and oxalate oxidase production. *A. gramineus* developed SSR in field and controlled

environments. Sclerotinia stem rot was not observed on *P. glaucum* in field environments. The disease developed on *P. glaucum* and *S. italica* in controlled environments, and severity increased with decreasing temperature and wounding. *J. inflexus*, *C. flagellifera*, and *I. cernua* developed no or minor symptoms of SSR in field and controlled environments. Mycelia penetrated *A. gramineus* leaves 24 h after inoculation (HAI) and *P. glaucum* at 48 HAI, but did not penetrate *J. inflexus* at 24, 48, or 96 HAI. Oxalate oxidase was not detected in inoculated leaves of these ornamental graminoids. The results illuminate *S. sclerotiorum*'s interactions with monocots and broaden the understanding of SSR resistance in ornamental grasses.

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic fungal plant pathogen that causes significant economic damage to a wide range of plant species including vegetables, legumes, oil seed crops, and ornamental flowering plants (Gleason et al. 2009; Hartman et al. 2015; Harveson et al. 2016; Koike 2007). The disease is commonly known as Sclerotinia stem rot (SSR) or white mold. Although symptoms vary among hosts, infection typically progresses into the plant stem, resulting in wilt and death of all parts distal to the infection. Sclerotia produced by *S. sclerotiorum* can survive many years in soil and may either germinate as mycelia or produce apothecia capable of releasing large numbers of airborne ascospores (Bolton et al. 2006; Schwartz and Steadman 1978). The wide host range of *S. sclerotiorum* combined with its ability to produce long-term survival structures and disseminate airborne ascospores make SSR a challenging disease to manage.

In ornamental landscape plantings, *S. sclerotiorum* causes stem rot, wilt, and death of many common herbaceous ornamental plants including *Zinnia* spp., *Petunia* spp., *Antirrhinum* spp., *Tagetes* spp., and *Salvia* spp. (Boland and Hall 1994; Farr and Rossman 2017). In the Midwestern United States, plant death often occurs in mid-to-late summer when replacement plants are no longer available. This results in large areas of dead plants or bare soil, which is an unacceptable condition for high value, high visibility landscape beds.

A potential disease management option for landscape managers is to choose plant species that resist infection by *S. sclerotiorum*. Unfortunately, ornamental plants are not commonly tested or bred for resistance to SSR, and the complete host range of *S. sclerotiorum* is not well defined. Over 400 species of plants from 75 families are reported to be susceptible to *S. sclerotiorum* (Boland and Hall 1994;

Farr and Rossman 2017). The majority of landscape ornamentals susceptible to *S. sclerotiorum* are herbaceous dicotyledons from numerous plant families (Daughtrey et al. 1995; Gleason et al. 2009). Grabowski and Malvick (2015) demonstrated that five commonly grown dicotyledonous herbaceous ornamentals that do not appear on the host lists of *S. sclerotiorum* were susceptible to infection in field and controlled environments. In contrast, monocotyledonous ornamental plants in the Araceae and Cannaceae varied in susceptibility to *S. sclerotiorum*, with several highly resistant species identified (Grabowski and Malvick 2017). Ornamental grasses comprise another group of potentially resistant plants that have not been tested for resistance or susceptibility and do not appear on host lists for *S. sclerotiorum*.

Of the five families of monocotyledonous plants listed as hosts to *S. sclerotiorum*, the only graminoid family included is the Poaceae (Boland and Hall 1994). The two most recently published host lists do not agree on which members of the Poaceae are hosts. Boland and Hall (1994) reported that plants from nine genera of Poaceae are hosts of *S. sclerotiorum*, whereas Farr and Rossman (2017) listed plants in eight genera as hosts. The genera *Zea*, *Sorghum*, *Setaria*, and *Triticum* occur on both host lists. Although corn, sorghum, and wheat are listed as hosts, *S. sclerotiorum* is not considered a pathogen of importance in these crops, despite their use in locations where environmental conditions frequently favor development of SSR (Bockus et al. 2010; Warren and Frederiksen 1986; White and Munkvold 2016).

Species in several genera of Poaceae are listed as hosts of *S. sclerotiorum* due to infection of grain in storage, but it is unclear if other plant parts can also be infected (Hysek et al. 1986; Miclaus et al. 1988; Richardson 1990). Plants within many genera of Poaceae appear on published disease indices that do not include a description of symptoms or severity of the infection (Anonymous 1960; Connors 1967; Galloway 1935; Poole 1922). Only the reference for *Setaria viridis* contains a description of disease signs and symptoms (Burke et al. 1957). Thus, the extent of *S. sclerotiorum*'s infection capabilities within graminoids remains unknown.

Ornamental grasses in the Poaceae, Cyperaceae, Juncaceae, and Acoraceae are available to landscape managers and valuable in ornamental landscape beds. None has been tested for resistance or susceptibility to *S. sclerotiorum*. The objectives of this study were to (i) compare the susceptibility of select ornamental grasses from these four families to *S. sclerotiorum* under different environment conditions, (ii) determine if temperature or wounding affect disease development, and (iii) characterize how ornamental graminoids respond to

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inoculation by examination of fungal growth and oxalate oxidase production in leaves. These studies expand knowledge of this pathogen's interactions with monocots and provide information on relative resistance of ornamental grasses for use in landscape beds infested with *S. sclerotiorum*.

Materials and Methods

Isolate preparation. Three isolates of *S. sclerotiorum* from diverse hosts were arbitrarily chosen for this study. Isolate ORN010 was isolated from a petunia plant in Chaska, MN; FC002 was isolated from a soybean plant in Renville County, MN; and VEG001 was isolated from green beans sold as produce in St. Paul, MN. All isolates were used in field studies, but only ORN010 was used in the growth chamber because previous studies demonstrated no difference in virulence or host × isolate interactions among the isolates (Grabowski and Malvick 2015, 2017). Isolates were grown and stored as described previously (Grabowski and Malvick 2015).

Plant materials. Ornamental grasses widely available to landscape managers in the Midwestern United States and representing diverse families and genera were selected for these studies. These included cultivars of species in six genera of graminoids from four families (Table 1). Seed of *Pennisetum glaucum* and *Setaria italica* (Johnny's Selected Seeds, Fairfield, Maine) were planted in 10.2-cm-square pots containing a composted bark potting mix (Sungro Horticulture, Agawam, MA) and maintained in a greenhouse with 12 h light at 25°C and 12 h darkness daily at 20°C. Seedlings were fertilized by watering pots to saturation 7 days after emergence with 20-20-20 soluble fertilizer (Everris NA, Inc., Dublin, OH), and Osmocote 14-14-14 (Everris NA, Inc.) was added to pots 2 weeks after emergence at 0.005 g/cm³. Plants were grown for approximately 10 weeks until 8–10 fully expanded leaves were present. *Juncus inflexus*, *Isolepis cernua*, *Acorus gramineus*, and *Carex flagellifera* were purchased as transplants in 10.2-cm-square pots (Linder's Greenhouses Inc., Roseville, MN). *Zinnia elegans* × *angustifolia* 'Profusion White' transplants (Linder's Greenhouses Inc.) were included in trials as a susceptible control. All plants were maintained in a greenhouse with 12 h light at 25°C and 12 h darkness daily at 20°C prior to planting in field trials.

Field studies of disease susceptibility. Studies were arranged in a randomized complete block design with six blocks and conducted at two field locations containing Waukegan silt loam in Falcon Heights, MN in 2011. Plants were transplanted into the field plots at one location on 8 June and at the other location on 14 June. Experimental units were 107 × 107 cm plots containing nine plants arranged in three rows of three plants each, with 35 cm spacing between plants. One row of Profusion Cherry zinnias was planted as a border around each block to help maintain even moisture within the plots and reduce edge effects. Plants were watered daily for 20 min with an oscillating sprinkler to promote establishment prior to inoculation. Osmocote 14-14-14 (340 kg/ha) was applied to the plots 2 weeks after planting.

Plots were inoculated with a mixture of equal quantities of sorghum seed colonized individually by the three isolates of *S. sclerotiorum* (Grabowski and Malvick 2015). The inoculum mixture was spread evenly over the plant canopy at 86.5 liters/ha on 4 August 2011 when the susceptible Profusion White zinnia had grown to achieve canopy closure, and again on 11 August. Irrigation was applied for 5 min immediately after inoculation and subsequently applied three times daily for 10 min for the duration of the study. Daily maximum and minimum temperatures were 22.7 to 29°C and 15.5 to 23°C, respectively.

Disease severity and incidence were recorded every 14 days after inoculation (DAI) until plants were killed by frost. Disease incidence was recorded as the number of plants per plot that were infected with *S. sclerotiorum* based on signs and symptoms. Symptomatic tissue from any plant with unidentifiable symptoms was surface sterilized and plated on 1/2× potato dextrose agar (PDA, Becton, Dickinson and Company, Franklin Lakes, NJ) to confirm infection by *S. sclerotiorum*. Disease severity was recorded as percent plant canopy missing within plots due to plant death from SSR. The area under disease progress curve (AUDPC) was calculated (Madden et al. 2007) for a 28-day period for field 2 and a 42-day period for field 1. The difference in period of observation for the two fields was the result of planting date differences and an early killing frost.

Disease susceptibility at different temperatures in controlled environments. The plant entries (Table 1) were grown as described above and evaluated for resistance in a growth chamber at 13, 16, 19, and 22°C. Experiments were arranged in a randomized complete block design with three blocks, and one plant per block. Sclerotia of *S. sclerotiorum* isolate ORN010 were placed on 1/2× PDA 5 days prior to the experiment and incubated at 22°C to promote mycelial growth. An 8 mm square of 1/2× PDA containing the leading edge of the fungal colony was secured to a lower stem of each plant with a sterile toothpick inserted 0.6 cm into the stem. Mock inoculated plants were treated in a similar fashion using sterile 1/2× PDA. Three 4- to 6-week-old *Phaseolus vulgaris* 'Provider' plants grown from seed in 10.2-cm-square pots were included as susceptible checks at each temperature. Plants were inoculated by placing the fungus on cut stems as described previously (Grabowski and Malvick 2015). Soil was thoroughly watered, then each plant was misted with water until run-off, covered with a clear plastic bag, and placed in a growth chamber (Environmental Growth Chambers, Chagrin Falls, OH) set to one of the four temperatures with a 12-h photoperiod and 90% relative humidity. Bags were removed 3 DAI. At 28 DAI, plants were cut at soil level, bisected vertically, and internal and external lesion length was measured. The experiment was performed twice at each temperature.

Disease susceptibility in response to wound inoculation. *A. gramineus*, *P. glaucum*, and *J. inflexus* were selected to represent susceptible, intermediate, and resistant responses to SSR, respectively. Plants were evaluated to determine the effect of wounding on infection by *S. sclerotiorum*. The experiment was arranged in a randomized complete block design with three blocks, one plant per block, and was conducted twice. Treatments included basal stem inoculation with or without wounding, leaf inoculation with or without wounding, and mock inoculated control. Basal stem inoculations were conducted as described above, with or without wounding the stem with a sterile toothpick. The agar plug was secured to the stem with Parafilm (Bemis Company, Inc., Oshkosh, WI) in wounded and nonwounded plants. Leaves were wounded by removing the distal half of a fully expanded leaf with a sterile razor blade; and then an agar plug containing *S. sclerotiorum*, prepared as described above, was placed on the cut edge. In nonwounded leaves, the agar plug was placed on the adaxial surface at the midpoint of the leaf. In both wounded and nonwounded leaves, the agar plug was attached with Parafilm. Mock inoculated controls were wounded at the leaf and stem as described above, and sterile 1/2× PDA was applied. Because *J. inflexus* lacks true leaves, the plants were wounded at the base of the stem as described above or the stem was inoculated 7.5 cm below the apical point with or without wounding. Plants were incubated for 72 h in a plastic bag following inoculation as described above and

Table 1. Ornamental grasses evaluated for susceptibility to *Sclerotinia sclerotiorum*

Scientific name	Cultivar	Common name	Family
<i>Acorus gramineus</i> Sol. ex Aiton	Ogon	Sweet Flag	Acoraceae
<i>Carex flagellifera</i> Colenso	Toffee Twist	Sedge	Cyperaceae
<i>Isolepis cernua</i> (Vahl) Roem. & Schult. (Syn. <i>Scirpus cernuus</i>)	Fiber Optic Grass	Low Bulrush	Cyperaceae
<i>Juncus inflexus</i> L.	Blue Mohawk	Reed	Juncaceae
<i>Pennisetum glaucum</i> (L.) R. Br. (Syn. <i>Cenchrus americanus</i>)	Purple Majesty	Millet	Poaceae
<i>Setaria italica</i> (L.) P. Beauv.	Lime Light Spray	Foxtail millet	Poaceae

maintained at 13°C in a growth chamber with a 12-h photoperiod and 90% RH. Lesion length and number of leaves killed due to infection was determined 28 DAI. The leading edge of the infection from plants with uncharacteristic symptoms was plated on 1/2× PDA and incubated 14 days at 22°C to determine if the pathogen was present.

Microscopic examination of leaf response to inoculation. *A. gramineus*, *P. glaucum*, and *J. inflexus* were selected to represent susceptible, intermediate, and resistant responses to SSR, respectively. Plants were grown in the greenhouse as described above. Fully expanded leaves were harvested, and 6-cm sections were cut from the middle of leaves with a sterile razor blade and placed on wet, sterile filter paper (Whatman International, Maidenstone, England) in a sterile 100 × 15 mm petri dish. An agar plug containing *S. sclerotiorum* isolate ORN010, prepared as described above, was placed at the center of each leaf segment on the adaxial surface. Mock inoculated controls received sterile 1/2× PDA. Petri dishes were sealed with Parafilm and incubated at 22°C with 10 h light and 14 h darkness. To determine if *S. sclerotiorum* produced infection cushions on nonplant surfaces, an agar plug of isolate ORN010 was also placed on a glass microscope slide, a glass slide wrapped in colored tape (Fisherbrand, Houston, TX), and a glass slide covered with colored construction paper (Wexford, Deerfield, IL) saturated with Millipore filtered water. The slides with inoculated nonplant surfaces were sealed in petri dishes and incubated as described above.

Leaves and inoculated nonplant surfaces were examined at 24 and 48 HAI. *J. inflexus* leaves were also examined at 96 HAI because no evidence of penetration was observed earlier. Agar plugs were removed from leaves and the inoculation site was examined with a Nikon SMZ 18 stereomicroscope (Nikon Corp., Tokyo, Japan) to determine the presence or absence of infection cushions. The section of leaf below the inoculation site was sectioned with a microtome. The cross sections were submerged in lactophenol blue stain (Sigma Aldrich, Inc., St. Louis, MO) for 1 min and then in water for 1 min prior to examination with a Nikon Eclipse NiU light microscope to observe the extent of fungal growth and infection.

Examination of oxalate oxidase activity in inoculated leaf tissue. *A. gramineus*, *P. glaucum*, *J. inflexus*, *C. flagellifera*, and *I. cernua* were grown as described above. *S. italica* was not included in the study due to insufficient quantities of seed. Wheat (*Triticum aestivum* ‘Morocco’) and barley (*Hordeum vulgare* ‘Bowman’) were used as positive controls. Seed of wheat and barley were planted in 10.2-cm-square pots, grown as described above for *P. glaucum* and *S. italica*. Fully expanded leaves were harvested from all plant entries. Eight 6-cm-long sections were cut from the middle of the leaves with a sterile razor blade and placed on wet filter paper in a petri dish as described above. An agar plug containing isolate ORN010, prepared as described above, was placed at the center, adaxial surface of each leaf segment. Mock inoculated controls received only sterile 1/2× PDA. Petri dishes were sealed with Parafilm (Bemis Company, Inc.) and incubated at 22°C for 1, 4, 6, or 24 h, with 10 h light and 14 h darkness. One inoculated and one mock inoculated leaf segment were inspected per time interval. Agar plugs were removed and leaf tissue was placed into 1.2-ml glass vials containing 1 ml of oxalate oxidase detection buffer (Dumas et al. 1995; Orshinsky et al. 2012) for 1 h at 22°C. Then leaves were removed and examined for dark blue staining indicative of oxalate oxidase activity with a Nikon Eclipse NiU light microscope. This experiment was conducted twice.

To determine if the cuticle interfered with detection of oxalate oxidase, leaf segments of *J. inflexus*, *I. cernua*, *C. flagellifera*, *A. gramineus*, *P. glaucum*, and wheat were harvested, inoculated, and incubated as described above. After 48 h, one leaf per plant entry was placed into the oxalate oxidase detection buffer for 1 h and examined as described above. Another leaf segment per plant entry was placed in a glass vial containing undiluted xylene and incubated for 24 h to remove the cuticle (Hilu 1984). Leaves were removed from the xylene, dried with a clean paper towel, placed into the oxalate oxidase detection buffer for 1 h, and examined as described above. This experiment was conducted three times.

Statistical analysis. Data from field experiments and wound inoculation trials were right skewed, not normal, and treatment variance was not equal (JMP Pro 13 software, SAS Institute Inc., Cary, NC). This could not be corrected with standard data transformations, and therefore nonparametric methods were used for data analysis. Friedman’s test was used to calculate a χ^2 value for the model and a *P* value for treatments. Treatments with a *P* value less than or equal to 0.01 were compared using Wilcoxon’s rank testing to determine significance of differences between treatments. Data from the variable temperature study were normally distributed, and variances were determined to be homogeneous between repeated experiments using Hartley’s Fmax test. Data from replicate experiments were combined and analyzed with ANOVA. Tukey’s honestly significant difference was used to separate means (JMP Pro 13 software, SAS Institute Inc.).

Results

Susceptibility of ornamental grasses to Sclerotinia stem rot in field environments. Disease incidence and severity 14 DAI for the susceptible control Profusion White zinnia averaged across both fields were 94 and 39%, respectively, indicating favorable conditions for SSR development (data not shown). *J. inflexus*, *I. cernua*, *C. flagellifera*, and *P. glaucum* were symptom free throughout the field study. *A. gramineus* was the only grass to develop symptoms of infection under the field conditions. Elongate tan lesions developed on the rhizomes, infection spread into leaf clusters, and leaves turned tan and died. The AUDPC for disease incidence and severity for all grasses was significantly lower than the susceptible zinnia control (Table 2). *S. italica* plants did not tolerate transplanting and died shortly after planting in the field.

Influence of temperature on disease susceptibility in a controlled environment. Plants of the susceptible check, *P. vulgaris*, developed stem rot and died by 10 DAI at all temperatures, indicating favorable conditions for SSR in the growth chamber (data not shown). Death of *P. vulgaris* and stem lesion length in *J. inflexus*, *C. flagellifera*, and *I. cernua* were unaffected by temperature (Figs. 1 and 2). In contrast, lesion length in *A. gramineus*, *P. glaucum*, and *S. italica* varied with temperature, and the effect of temperature on disease severity varied by plant entry (Figs. 1 and 2). Lesion length for *P. glaucum* and *S. italica* increased greatly as temperature decreased, and was significantly greater at 13°C than at all other temperatures (Figs. 1 and 2). Lesion length for *A. gramineus* did not differ between 13, 16, and 19°C, but was significantly lower at 22°C (Figs. 1 and 2).

J. inflexus, *C. flagellifera*, and *I. cernua* were resistant to *S. sclerotiorum* at all temperatures (Figs. 1 and 2). Internal and external lesion length for *J. inflexus*, *C. flagellifera*, and *I. cernua* did not differ significantly between inoculated and mock inoculated plants at any temperature (Table 3). Small, brown lesions (<1.0 cm) were observed on 8 and 21% of inoculated *C. flagellifera* and *J. inflexus* plants, respectively. Presence of *S. sclerotiorum* in symptomatic tissue was confirmed by isolation, and a small sclerotium was present in 8% of the stems from these species (Supplementary Fig. S1). No signs

Table 2. Area under disease progress curve (AUDPC) for incidence and severity of Sclerotinia stem rot in field-inoculated ornamental grasses

	Incidence AUDPC		Severity AUDPC	
	Field 1	Field 2	Field 1	Field 2
<i>Carex flagellifera</i>	0 c ^z	0 c	0 b	0 b
<i>Juncus inflexus</i>	0 c	0 c	0 b	0 b
<i>Pennisetum glaucum</i>	0 c	0 c	0 b	0 b
<i>Isolepis cernua</i>	0 c	0 c	0 b	0 b
<i>Acorus gramineus</i>	211 b	146 b	22 b	7 b
<i>Zinnia elegans</i> × <i>angustifolia</i>	3344 a	2048 a	2132 a	942 a

^z AUDPC was calculated for a 28-day period for field 2 and a 42-day period for field 1. All statistical comparisons are among entries within each field. Values with the same letter within a column are not significantly different at $\alpha = 0.001$ by Wilcoxon’s rank testing.

or symptoms were observed in inoculated *I. cernua* plants, and the pathogen was not isolated.

Symptoms of SSR developed on *A. gramineus*, *P. glaucum*, and *S. italica* at all temperatures. Although SSR resulted in plant death of the susceptible check *P. vulgaris* by 10 DAI, SSR in these ornamental graminoids did not progress to plant death 28 DAI. Stem lesion length of *P. glaucum* and *S. italica* was greater than mock inoculated controls at all temperatures (Table 3). Symptoms of infection in *P. glaucum* and *S. italica* plants included dark brown to black elongate stem lesions that extended into the pith. Pith cells were degraded, creating a hollow elongate cavity lined with white mycelia and containing sclerotia (Supplementary Fig. S2). Internal discoloration of the pith extended beyond visible lesion length on the epidermis of the stem of *P. glaucum* and *S. italica* in both directions at 13 and 16°C (data not shown). Stem lesion length on *A. gramineus* was greater than mock inoculated controls at 13, 16, and 19°C (Table 3). Although symptoms of infection were visible at 22°C, lesion length did not significantly differ from mock inoculated controls at this temperature (Table 3). An elongate tan lesion developed on *A. gramineus* rhizomes, infection spread into leaf clusters, and leaves turned tan and died (Supplementary Fig. S3). Mycelia and sclerotia were observed in infected leaf clusters of *A. gramineus*.

Fig. 1. External lesion length on stems of ornamental grasses inoculated with *Sclerotinia sclerotiorum* and incubated at four temperatures. Values with the same letter within a plant entry are not significantly different at $\alpha = 0.01$ by Tukey's honestly significant difference.

Fig. 2. Internal lesion length in stems of ornamental grasses inoculated with *Sclerotinia sclerotiorum* and incubated at four temperatures. Values with the same letter within a plant entry are not significantly different at $\alpha = 0.01$ by Tukey's honestly significant difference.

Influence of wounding on disease development and resistance.

Wounding was required for infection of the most resistant plant entry, *J. inflexus*, and it increased disease severity in the moderately resistant plant entry *P. glaucum*, but did not affect severity in the susceptible plant entry *A. gramineus*. Necrosis developed in inoculated leaves and stems of *A. gramineus*, regardless of wound treatment, and lesion length did not differ between wounded and nonwounded plants (Table 4). External stem lesion length and leaf lesion length of inoculated *P. glaucum* did not differ between wounded and nonwounded plants (Table 4). Infection progressed into stem pith tissue only in wounded stems (Table 4), resulting in significantly greater internal lesion length in wounded *P. glaucum* stems. *J. inflexus* developed a measurable necrotic lesion only when wounded. Lesion length on apical wounded stems was significantly greater than nonwounded and mock inoculated stems. Lesion length on basal wounded stems did not differ significantly from nonwounded or mock inoculated plants. In *J. inflexus*, *S. sclerotiorum* was isolated from 100% of apical wounded stems and 33% of basal wounded stems (Table 4).

Microscopic examination of the infection site. *S. sclerotiorum* initiated the infection process on the resistant, intermediate, and susceptible plant entries, *J. inflexus*, *P. glaucum*, and *A. gramineus*, respectively, as indicated by development of multiple infection cushions on leaves of all inoculated plants at 24 and 48 HAI (Supplementary Fig. S4). Infection cushions did not develop on nonplant surfaces, i.e., glass slides, slides with lab tape, or slides with paper.

Table 3. External stem lesion length on six ornamental grasses 28 days after inoculation with *Sclerotinia sclerotiorum* and incubation at 13, 16, 19, and 22°C

	Stem lesion length (cm)			
	13°C	16°C	19°C	22°C
<i>Acorus gramineus</i>	2.7 a ^y	2.0 a	1.8 a	0.5 bc
<i>Setaria italica</i>	2.4 a	1.6 ab	1.3 ab	1.1 a
<i>Pennisetum glaucum</i>	2.7 a	1.1 b	0.9 b	0.7 ab
<i>Juncus inflexus</i>	0.1 b	0.0 c	0.6 bc	0.4 bc
<i>Carex flagellifera</i>	0.1 b	0.0 c	0.0 c	0.2 c
<i>Isolepis cernua</i>	0.0 b	0.0 c	0.0 c	0.0 c
Mock inoc. control ^z	0.00 b	0.00 c	0.00 c	0.00 c

^y Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$ by Tukey's honestly significant difference.

^z All mock inoculated controls had the same value. Although statistical analysis was conducted on individual mock inoculated entries, mock inoculated data are displayed as one representative entry.

Table 4. Lesion length on wounded or nonwounded leaves and stems of *Juncus inflexus*, *Acorus gramineus*, and *Pennisetum glaucum* 28 days after inoculation with *Sclerotinia sclerotiorum* and incubation at 13°C

Treatment	Lesion length (cm)			
	<i>Juncus inflexus</i>	<i>Acorus gramineus</i>	<i>Pennisetum glaucum</i>	
			External	Internal
Leaf nonwounded	0.0 b ^x	12.8 b ^y	0.0 a ^x	0.0 a ^x
Leaf wounded ^z	5.3 a	11.6 b	0.0 a	0.0 a
Stem nonwounded	0.0 b	32.1 c	0.9 b	0.0 a
Stem wounded	0.1 b	25.0 bc	1.9 b	3.0 b
Noninoculated control	0.0 b	0.0 a	0.0 a	0.0 a

^x Values in a column followed by the same letter are not significantly different at $\alpha = 0.001$ by Wilcoxon's rank testing.

^y Values in a column followed by the same letter are not significantly different at $\alpha = 0.01$ by Wilcoxon's rank testing.

^z Leaf inoculation occurred at the midpoint of a true leaf of *A. gramineus* and *P. glaucum*, and 7.5 cm below the apical tip of *J. inflexus* stems.

The ability of *S. sclerotiorum* to infect leaves and the rate of infection varied among plant entries. Infection and colonization of epidermal cells, mesophyll cells, and elaioplasts of *A. gramineus* were observed at 24 HAI (Supplementary Fig. S5), but leaf sections were too degraded to be sectioned at 48 HAI. Infection cushions developed on the surface of *P. glaucum* leaves 24 HAI, and penetration and colonization of epidermal and mesophyll cells was observed 48 HAI. No penetration of *J. inflexus* tissue was observed at 24, 48, or 96 HAI, although multiple infection cushions were present at all three time points. By 96 HAI, the infection cushions on inoculated *J. inflexus* were degraded, flattened, and lacked structure; and the cuticle, mesophyll cells, and sclerenchyma below the infection cushions were discolored brown but remained intact and turgid.

Examination of oxalate oxidase activity in inoculated leaf tissue. Dark staining indicative of oxalate oxidase production was observed at the edge of lesions in the positive controls, barley and wheat, at 24 and 48 HAI. With the aid of magnification, staining was observed in cell walls and stomata around the lesion edge in these plants. No staining was observed macroscopically or microscopically at the inoculation point of *A. gramineus*, *J. inflexus*, *I. cernua*, *P. glaucum*, or *C. flagellifera* at any time point. Cuticle removal with xylene did not affect lesion development or oxalate oxidase staining.

Discussion

This study demonstrates a range of susceptibility and resistance responses to *S. sclerotiorum* in inoculated ornamental graminoids. Although none of the ornamental graminoids in this study appear on the host list, *S. sclerotiorum* was able to infect and create a lesion in five of the six plant entries under some experimental conditions. As a result, *A. gramineus*, *P. glaucum*, *S. italica*, *C. flagellifera*, and *J. inflexus* should be added to the U.S. National Fungal Collections Fungal Database (USDA, ARS) as hosts for *S. sclerotiorum*, as this list represents all reported fungus–host associations. *I. cernua* is the only ornamental graminoid included in these studies that did not become infected with *S. sclerotiorum* under any conditions included in this study.

Useful levels of resistance were identified in several ornamental graminoids. All ornamental graminoids tested exhibited much lower SSR severity compared with susceptible controls zinnia and common bean. *I. cernua* was immune to infection under all conditions examined in the study. *C. flagellifera* and *J. inflexus* were considered resistant due to their ability to limit the infection to a few centimeters around the infection site, resulting in minimal overall damage to the plants. *P. glaucum* and *S. italica* demonstrated moderate resistance to SSR, with wounding and temperature playing a significant role in disease development. *A. gramineus* was susceptible to SSR under all conditions tested, but infection never progressed to plant death as was observed in susceptible controls.

This is the first histological study describing attempted infection of graminoids by *S. sclerotiorum*. Studies have previously shown that *S. sclerotiorum* forms domed infection cushions with multiple penetration hyphae positioned perpendicular to the host surface to initiate an infection (Lumsden and Dow 1973; Lumsden and Wergin 1980). In this study, *S. sclerotiorum* formed infection cushions on detached leaves of the three ornamental graminoids examined, but not on the mock leaf surfaces. This indicates that *S. sclerotiorum* recognized the graminoids as potential hosts. The recognition, however, did not lead to successful infection and disease development in all of the species examined.

Three different plant responses to the initial recognition and attempted infection by *S. sclerotiorum* were observed in this study. First, penetration and infection was complete and rapid. *A. gramineus* became infected 24 h after inoculation (HAI), indicating a lack of defense mechanisms to prevent initial infection. This is similar to what was described for infection of susceptible bean by *S. sclerotiorum* (Abawi et al. 1975; Lumsden and Dow 1973). In the second type of response, infection by *S. sclerotiorum* was delayed but not prevented, as was observed for *P. glaucum*. Third, infection was prevented, as observed with *J. inflexus*, which prevented penetration through the 96-h viable lifespan of the infection cushion. This result and the fact that infection occurred after wounding suggests that

J. inflexus possesses structural defense mechanisms that inhibit the ability of *S. sclerotiorum* to penetrate plant tissue and initiate infection. In addition, discoloration of the cuticle, epidermal, and mesophyll cells occurred at the infection site in *J. inflexus* at 96 HAI, indicating potential production of plant defense compounds that inhibit further infection and/or disease development. For example, these could include production phenols, phytoalexins, pathogenesis related proteins, cell wall degrading enzymes, and proteins associated with the oxidative burst as has been reported in resistance responses to *S. sclerotiorum* in other hosts (Bazzalo et al. 1985; Hegedus et al. 2008; Prats et al. 2003; Urdangarín et al. 1999; Wang et al. 2012; Zhao et al. 2007).

Production of oxalate oxidase is a known defense response against fungal pathogens, including *S. sclerotiorum* (Hurkman and Tanaka 1996; Zhou et al. 1998). Oxalic acid has been identified as an important pathogenicity factor for *S. sclerotiorum*, and transgenic introduction of the oxalate oxidase gene into susceptible crops has increased resistance to *S. sclerotiorum* (Cessna et al. 2000; Cober et al. 2003; Donaldson et al. 2001; Godoy et al. 1990; Guimaraes and Stotz 2004; Hu et al. 2003; Marciano et al. 1983; Noyes and Hancock 1981). In this study, oxalate oxidase production was not detected in response to inoculation with *S. sclerotiorum* in any of the ornamental graminoids, although it was produced in the wheat and barley used as positive controls. These results indicate that resistant ornamental grasses utilize defense mechanisms other than oxalate oxidase against *S. sclerotiorum*.

All ornamental graminoids that became infected with *S. sclerotiorum* in this study reduced colonization of host tissue and limited disease development compared with susceptible controls, i.e., zinnia and common bean. As noted above, multiple defense mechanisms were likely utilized by the different plant species to accomplish this. In addition, the fact that disease development was not equally influenced by temperature in the different plant species also suggest that different temperature-dependent defense mechanisms likely affect tissue colonization and disease progression in the graminoid species examined. Disease severity for the two species from the Poaceae, *S. italica* and *P. glaucum*, had an inverse relationship with temperature. Disease severity in *A. gramineus* was lower at 22°C compared with the consistent severity measured at 13, 16, and 19°C. However, temperature did not affect disease severity in *C. flagellifera* or *J. inflexus*. These results indicate that different temperature-dependent defense mechanisms may affect tissue colonization and disease progression in the Poaceae plant entries and *A. gramineus*, and that one or more temperature-independent defense mechanisms may restrict colonization of host tissue in *C. flagellifera* and *J. inflexus*.

The ornamental graminoids with the highest levels of resistance identified in this study may be useful in ornamental landscape plantings where SSR is a persistent problem. Furthermore, some of the likely different resistance mechanisms detected in the ornamental graminoids challenged by *S. sclerotiorum* are potentially novel and may be useful in breeding for resistance to this pathogen. Further research is needed to characterize and identify these resistance mechanisms. Novel resistance mechanisms could potentially be utilized to increase resistance to SSR in susceptible hosts, perhaps through use of transgenic methods as was done with oxalate oxidase (Cober et al. 2003; Donaldson et al. 2001; Hu et al. 2003). This work advances our understanding of the host–pathogen interactions between *S. sclerotiorum* and graminoids.

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