Exploring the host range of *Sclerotinia sclerotiorum* in herbaceous ornamental plants

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Abstract

Sclerotinia sclerotiorum is a necrotrophic fungal plant pathogen known to infect over 400 species of plants from 75 families. Many popular herbaceous flowering plants are susceptible. Infested flower beds often suffer significant plant loss each year, reducing their ornamental value significantly. Identification of disease resistant plants would be a useful management tool. The objective of this study was to evaluate species of herbaceous ornamental plants with no reported history of white mold susceptibility for potential resistance to *S. sclerotiorum.* Plant species included *Portulaca grandiflora, Pentas lanceolate, Scaevola aemula, Impatiens hawkeri, I. walleriana, Caladium ×hortulanum, Canna ×generalis*, and *Colocasia esculenta,* and the ornamental grasses *Pennisetum glaucum, Setaria italica, Juncus inflexus, Carex flagellifera, Isolepsis cernua*, and *Acorus gramineus*. *Zinnia elegans x angustifolia* 'Profusion White' served as a susceptible control. Disease response was evaluated under field conditions and in controlled environments. Direct inoculation of below ground storage organs of some species was conducted to determine the pathogen's ability to survive and grow in these plant parts. The effect of temperature and wounding was evaluated in ornamental grasses through inoculation of plants grown at 13, 16, 19, or 22ºC with and without wounding. Detached leaves of representative ornamental grasses were inoculated and stained to determine the pathogen's ability to penetrate leaf tissue. In field trials, disease incidence was quantified as the number of plants per bed visibly infected with *S. sclerotiorum* based on signs and symptoms and disease severity was recorded as percent canopy missing due to death of plants within the bed. In controlled environment studies, lesion

length was used as a measure of disease severity. *Portulaca grandiflora, P. lanceolata,* and *S. aemula* were highly susceptible in a controlled environment but displayed reduced disease severity in field conditions compared to susceptible controls. *Impatiens hawkeri* and *I. walleriana* displayed abscission of diseased plant tissue as an unusual resistance response. *Caladium ×hortulanum* was susceptible to *S. sclerotiorum*. Petioles, leaves, and corms developed a watery soft rot. *Colocasia esculenta* was resistant to infection. *Sclerotinia sclerotiorum* infected only wounded or senescent tissue of this species and did not result in significant symptoms under any conditions. *Canna ×generalis* was partially resistant to the pathogen. Although canna petals were readily infected, infection of petioles was restricted to small necrotic lesions. Neither infection progressed to the main stem or resulted in plant death. *Acorus gramineus* was susceptible in field and growth chamber environments, regardless of wounding and temperature. *Juncus inflexus, C. flagellifera,* and *I. cernua* were resistant with minor to no symptom development in field and controlled environment studies. The reaction of *P. glaucum* and *S. italica* to inoculation varied under different conditions. Disease severity increased as temperature decreased and with wounding. Microscopy studies of ornamental grasses supported results from the field and growth chamber trials. Mycelial penetration of leaf tissue occurred 24 hours after inoculation (hai) for the susceptible entry *A. gramineus*, 48 hai for the species with intermediate resistance *P. glaucum,* and was not observed at 24, 48, or 96 hai for the resistant plant entry *J. inflexus*. This study indicates that *I. hawkeri, C. ×generalis, C. esculenta, J. inflexus, C. flagellifera,* and *I. cernua* have resistance to *S. sclerotiorum* and could be utilized in an integrated disease management program for infested landscape beds.

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Chapter 1

Literature Review

Sclerotinia sclerotiorum (Lib.) de Bary (1884) is an ascomycete in the Sclerotiniaceae. This fungal plant pathogen has worldwide distribution and causes diseases known as white mold, Sclerotinia stalk rot, cottony soft rot, watery pod rot, or Sclerotinia head rot (CABI 2017). Disease results in significant economic damage to a variety of crops including soybean, dry bean, canola, sunflower, and vegetables such as lettuce, carrot, and tomato (Bolton et al., 2006).

***Characteristics of* Sclerotinia sclerotiorum.** Like all members of the Sclerotiniaceae, *S. sclerotiorum* produces a sclerotial stroma (Bolton et al., 2006). Sclerotia serve as long term survival structures and form in response to low nutrient availability and restricted space. Sclerotia of *S. sclerotiorum* vary greatly in size and shape. Each sclerotium consists of a black melanized rind, three to four cell layers thick, and a light colored medulla of elongate cells embedded in a fibrillary matrix (Le Tourneau, 1979). *Sclerotinia sclerotiorum* is capable of forming scleotia from 0 to 30 ^{0}C and at pH of 2.5 to 9 (Le Tourneau, 1979). Cook et al. (1975) demonstrated sclerotia buried in the soil can survive over 3 years but survival is decreased with high soil moisture content, warm temperatures, or exposure at the soil surface. Sclerotia play a role in movement of the pathogen into new areas as they can be spread with contaminated seed, soil, and plant debris (Schwartz and Steadman, 1978a).

Sclerotia may germinate through myceliogenic germination in which hyphae emerge directly through cracks in the rind. Melanization of the rind inhibits myceliogenic germination, resulting in low rates of germination of mature sclerotia with an intact black rind (Huang et al., 1998). Light colored sclerotia germinate at a greater rate than darker sclerotia (Huang, 1985) indicating myceliogenic germination is increased if the rind is

not completely melanized. In addition, myceliogenic germination occurs if the rind is physically damaged or cracked by exposure to freezing temperatures or through wet and dry cycles (Huang et al., 1998). As a result, newly formed, mature sclerotia are likely to remain dormant until exposure to changes in moisture and temperature results in cracks in the rind that allow for myceliogenic germination (Huang et al., 1998).

Host root and seed exudates play a role in myceliogenic germination and infection of roots and seedlings. Sclerotia in soil located more than 2 to 4 cm away from carrot, bean, and sunflower roots did not germinate or initiate infection (Abawi and Grogan, 1975; Finlayson et al., 1989; Huang and Dueck, 1980). Sunflower seeds and roots adjacent to sclerotia (<2 cm) resulted in high levels of myceliogenic germination of sclerotia, infection, and death of plants (Huang and Hoes, 1980). In contrast, sclerotia placed next to bean seeds, alongside a bean stem at the soil line, or in the soil around bean roots failed to germinate or infect (Abawi and Grogan, 1975). If an exogenous source of nutrients, like a sterilized celery stalk, was placed between the bean plant and the sclerotia, however, mycelial germination and infection occurred (Abawi and Grogan, 1975). These results indicate that exudates from sunflower seeds and roots stimulate myceliogenic germination of sclerotia, and exudates from bean do not, but that exogenous nutrients can stimulate myceliogenic germination in the absence of stimulatory host exudates.

Sexual recombination may occur within the sclerotia resulting in production of apothecia; open cup-like ascocarps produced on a stalk or stipe. This is known as carpogenic germination. Continuous soil moisture is required for carpogenic germination (Abawi and Grogan, 1975). As a result, carpogenic germination is commonly observed

when soil is moist and shaded, for example when the crop canopy closes, in irrigation furrows, and close to the plant stem (Schwartz and Steadman, 1978a). Stipe formation originates in the medulla of the sclerotia. Stipes erupt through the rind, and grow phototropically towards the soil surface (Le Tourneau, 1979). Although sclerotia have been observed evenly distributed in the soil profile of conventionally tilled crops (Schwartz and Steadman, 1978a), stipe length averages 2-3 cm, and the majority of apothecia observed in field conditions originate from sclerotia within the upper 3 cm of the soil profile (Abawi and Grogan, 1979). Differentiation of the stipe into an apothecial disc occurs in response to light (Le Tourneau, 1979). Schwartz and Steadman (1978) recorded an average of two apothecia produced per sclerotia under field conditions. Each apothecium produced ascospores for an average of 9 days, releasing 2.32×10^6 ascospores. The ascospores are carried by wind to susceptible crops.

Abawi and Grogan (1979) reported the presence of ascospores from May through September in New York, and as early as April provided that snow had melted, moisture was present, and soil temperatures were above 10 ^{0}C. Ascospores require 16 to 72 hours of continuous moisture to germinate (Abawi and Grogan, 1979). Although ascospores will germinate in water alone, appresoria are only formed in the presence of a carbohydrate source (Abawi et al., 1975). As a result, ascospores cannot directly infect intact bean leaves without the presence of exogenous nutrients (Abawi and Grogan, 1975).

Infection. In many crops, ascospores first infect fallen petals or other senescent tissue, a readily available source of carbohydrates, and then proceed to infect leaves, stems, or fruit (Abawi and Grogan, 1975; Atallah and Johnson, 2004; Bradley et al.,

2006). Hyphae extend from the colonized senescent plant tissue and form a complex branched appresorium referred to as an infection cushion. Domed infection cushions consist of mounded mycelia with hundreds of penetration hyphae positioned perpendicular to the host surface (Abawi et al., 1975; Lumsden, 1979; Lumsden and Dow, 1973; Lumsden and Wergin, 1980). A mucilaginous compound adheres the cushion to the surface of the plant (Lumsden, 1979). The pinching of surrounding cell tissue, the visible depression in the cuticle seen after infection, and the fact that hyphae later cannot exit the plant cuticle but must use stomata all indicate that the initial penetration is physical not enzymatic (Abawi et al., 1975; Lumsden, 1979).

Lumsden and Dow (1973) describe two distinct forms of hyphae involved in the colonization of bean hypocotyls. Initially, large granular hyphae, known as infection hyphae, spread rapidly in all directions below the cuticle and progress more slowly through the cortex in an intercellular fashion. Degradation of pectic materials in host cell walls, changes in cell permeability, accumulation of fluids, water soaking, and host cell death can be observed in advance of infection hyphae. Ramifying hyphae, profusely branched hyphae with a smaller diameter, form behind the tip of the infection hyphae 12 to 24 hours after infection. Ramifying hyphae are associated with destruction of cell wall structure, can infect vascular tissue, and progress both inter and intracellularly. After extensive colonization of host tissue, ramifying hyphae grow through stomata or cracks in the epidermis to form superficial mycelia and sclerotia.

Pathogenicity factors. *Sclerotinia sclerotiorum* is a necrotrophic plant pathogen. In order obtain nutrition from host plants, *S. sclerotiorum* utilizes a variety of pathogenicity factors to infect and kill plant tissue. Pathogenicity factors include oxalic

acid (OA), cell wall degrading enzymes, and Nep 1-like proteins (NLPs). Oxalic acid was first isolated from infected plant tissue and cultures of *S. sclerotiorum* by De Bary (1886). Many studies have described a correlation between OA accumulation in infected plant tissue and severity of symptoms (Marciano et al. 1983; Noyes and Hancock 1981; Maxwell et al. 2007). Marciano et al. (1983) demonstrated that a weakly virulent strain of *S. sclerotiorum* produced only one third as much OA as a highly virulent strain. Godoy et al. (1990) demonstrated that mutants of *S. sclerotiorum* that cannot produce OA were nonpathogenic on bean leaves, stems, and pods, but exogenous application of calcium oxalate temporarily reinstated pathogenicity. Oxalic acid affects multiple aspects of the infection process; it reduces the pH of the apoplast to levels toxic to host cells (Cessna et al. 2000; Kim et al. 2007; Maxwell and Lumsden 1970), causes stomatal opening and wilt (Guimaraes and Stotz 2004; Noyes and Hancock 1981), facilitates activity of cell wall degrading enzymes (Favaron et al. 2004; Godoy et al. 1990; Marciano et al. 1983), represses the oxidative burst associated with resistance (Cessna et al. 2000), and stimulates programmed cell death (Dickman et al. 2001).

Sclerotinia sclerotiorum produces a wide range of cell wall degrading enzymes including xylanases, cellulases, pectinases, and polygalacturonases (Bolton et al. 2006; Favaron et al. 2004; Godoy et al. 1990; Marciano et al. 1983). These enzymes degrade structural components of host cell walls and facilitate intracellular colonization and nutrient acquisition. Many of the cell wall degrading enzymes depend upon the presence of OA to be effective. Godoy et al. (1990) demonstrated that OA- mutants of *S. sclerotiorum* were unable to cause infection in bean tissue even though levels of polygalacturonase, pectinmethylesterase, and cellulase were equal or greater than levels

in wild type infections. Marciano et al. (1983) found that a weakly virulent strains of *S. sclerotiorum* produced equal levels of cellulose, polygalacturonase, and xylanase but only one third of the oxalic acid of a highly virulent strain. Oxalic acid lowers the pH of the apoplast to levels that optimize activity of cell wall degrading enzymes while inhibiting the function of plant defense compounds (Cessna et al. 2000; Favaron et al. 2004; Marciano et al. 1983).

Dickman et al. (2001) demonstrated that programmed cell death (PCD) occurred in plants infected by *S. sclerotiorum* and that OA played an active role in initiating PCD through the hypersensitive response (HR). Another group of PCD elicitors known as Nep 1-like proteins (NLPs) were also identified in *S. sclerotiorum* (Dallal Bashi et al. 2010). Expression of two different NLPs was observed in necrotic tissue and at the leading edge of a *S. sclerotiorm* infection (Dallal Bashi et al. 2010). NLPs have been documented to trigger several plant defense signal mechanisms including ethylene, salicylic acid, and reactive oxygen species (Pemberton and Salmond 2004). Application of NLP protein to a plant cell results in necrosis through PCD (Pemberton and Salmond 2004). It is hypothesized that necrotrophic pathogens like *S. sclerotiorum* elicit PCD in order to facilitate nutrient acquisition from plant tissue.

***Population structure and diversity of* S. sclerotiorum.** Phenotypic and genotypic variation among isolates of *Sclerotinia sclerotiorum* has been reported. The fungus has been reported to vary for a number of traits including hyphal pigmentation (Garg et al. 2010), virulence (Aldrich-Wolfe et al. 2011; Garg et al. 2010), level of oxalic acid production (Durman et al. 2005), and temperature required to stimulate carpogenic germination (Garg and Barbetti 2010). High levels of genotypic variation have also been

recorded in populations of *S. sclerotiorum*. Through comparison of mycelial compatibility groups (MCG) and genetic markers, populations of *S. sclerotiorum* have been shown to be largely clonal with some recombination events occurring (Aldrich-Wolfe et al. 2010; Atallah et al. 2004; Carpenter et al. 1999; Cubeta et al. 1997; Kohli et al. 1992; Kohn et al. 1991; Kohn 1995). Populations in a specific region or crop are often diverse, containing many MCGs and a diversity of genotypes. Most populations however are dominated by a limited number of clones (defined as individuals with identical MCG and genotype) with most isolates falling into one to six clonal groups (Aldrich-Wolfe et al. 2010; Atallah et al. 2004; Kohli et al. 1992). Diversity is then represented by a large number of clones with only one or two representative isolates. Diversity of isolates may arise in a field through introduction of unique isolates as airborne ascospores or through introduction of sclerotia with contaminated seed or soil.

In addition, genotypic diversity in *S. sclerotiorum* can occur through genetic recombination during sexual reproduction. Multiple studies compared MCGs of sibling groups of ascospores in order to determine if genetic recombination occurred during sexual reproduction. Kohli et al. (1992) and Kohn et al. (1991) examined ascospore sibling groups from canola fields in Canada and found no evidence of recombination. In contrast, Atallah et al. (2004), Cubeta et al. (1997), and Kohn (1995) observed occasional ascospore sibling groups with multiple MCGs from isolates collected from potato, cabbage, and *Ranunculus*, respectively. These results indicate that *S. sclerotiorum* is homothallic and often reproduces through self-fertilization but is also capable of genetic recombination. Ford et al. (1995) demonstrated that isolates of *S. sclerotiorum* from compatible vegetative compatibility groups formed a stable heterokaryon and proposed

that heterokaryotic hyphae in sclerotia could result in genetic recombination and genetic variability between ascospore sibling groups. Despite observations of genetic and phenotypic variation in populations of *S. sclerotiorum*, host specificity has not been identified for any isolate (Aldrich-Wolfe et al. 2011; Atallah et al. 2004).

Host range. *Sclerotinia sclerotiorum* has a broad host range and is capable of infecting over 400 plant species from 75 families (Boland and Hall 1994; Farr and Rossman 2017). The epidemiology varies depending on which host plant is being infected. The infection cycle of commonly grown crops, of which *S. sclerotiorum* is a significant pathogen, is described below. The infection cycle of *S. sclerotiorum* in weed hosts, native plants, and crops with minor economic importance is less well understood.

White mold in sunflower. *Sclerotinia sclerotiorum* causes two distinct forms of disease in sunflower; basal or stalk rot, caused by myceliogenic germination of sclerotia at or below the soil line and seed or head rot caused by ascospore infection of the flower (Harveson et al. 2016). The root and hypocotyl of sunflower seedlings can be infected if sclerotia are located less than 2 cm away from germinating seed (Huang and Dueck 1980). Infection of mature root systems is initiated through lateral roots, then progresses into the lower stem and tap root (Huang and Hoes 1980). Infected stems and roots initially become water soaked, feeder roots slough off, the stem and taproot become hollow, and sclerotia form internally. The lower stem may be directly infected by sclerotia at or near the soil surface. Girdling of the lower stem and root rot results in wilt and death of the plant (Huang 1977; Huang and Hoes 1980). Huang and Hoes (1980) demonstrated that basal rot spreads between closely spaced plants due to mycelial growth between root systems.

Head rot occurs when ascospores initiate infection of the flower head. Ascospore infection occurs primarily through germination on and penetration of anthers (Pedraza et al. 2004). The infection progresses into the ovary and results in water soaked lesions on the receptacle. Infection degrades the quality of seed and results in sclerotia formation within the seed head. In severe cases the entire head rots, leaving dry fibrous plant material interspersed with sclerotia (Harveson et al. 2016). Ascospores can also infect leaves, stems, and buds if an exogenous source of carbohydrates, like fallen petals or wounded tissue, is present (Castaño et al. 1993). These infections can progress into stem tissue in the aerial part of the plant eventually girdling the stem. Similar to basal stem infection, girdling of the stem tissue results in wilt and death of all tissue distal to the infection (Castaño et al. 1993). Sclerotia produced on seed heads can contaminate seed lots and result in spread of the pathogen to previously uninfested fields (Harveson et al. 2016). Sclerotia produced in infected heads, roots, and stems are distributed through the field with post-harvest tillage practices and serve as inoculum for future crops (Harveson et al. 2016).

White mold in the Fabaceae. White mold is a significant disease problem of green bean, dry edible bean, and soybean where cool moist conditions occur during flowering (Hartman et al. 2015; Schwartz et al. 2005). In bean crops, infection occurs predominantly due to caropgenic germination of sclerotia in and near the field. Abawi and Grogan (1975) demonstrated that ascospores deposited on bean leaves could only initiate infection if wounds or petals were present on the leaf tissue. The fallen petals serve as a nutrient source for germinating ascospores and allow *S. sclerotiorum* to infect leaves, stems, and pods (Abawi and Grogan 1975; Abawi et al. 1975; Abawi and Grogan

1979). Infected tissue is initially water soaked but dries out and becomes bleached as the infection progresses. Pith tissue degrades and sclerotia often form within and on stems. Seeds can be infected with mycelia and sclerotia may form within or on the seed pod. Infection of roots does not occur in bean (Abawi and Grogan 1975).

White mold in canola, tobacco, and vegetable crops. Carpogenic germination of sclerotia and infection by ascospores play important roles in white mold of most susceptible crops. Infection of canola, potato, and tomato commonly occurs through ascospore infection of petals and senescent flowers similar to infection of bean (Atallah et al. 2004; Bradley et al. 2006; Letham et al. 1976). *Sclerotinia sclerotiorum* utilizes senescent or wounded leaf tissue to infect stems of lettuce, tobacco, and carrot (Abawi and Grogan 1979; Finlayson et al. 1989; Mila et al. 2001). Myceliogenic germination of sclerotia and infection of the lower stem can occur in these crops. Basal stem infection of lettuce and tomato occurs rarely (Abawi and Grogan 1979; Letham et al. 1976). In canola, 15% of plants were infected when sclerotia were placed adjacent to the stem at soil level (Huang and Dueck 1980). Although white mold is a common post-harvest rot problem in carrot (Koike 2007), Finlayson et al. (1989) were unable to initiate root infection of carrot through placement of sclerotia near the root. They demonstrated that infection of the taproot primarily occurred through infection of the foliage or crown followed by growth of *S. sclerotiorum* into root tissue.

White mold in ornamental plants. *Sclerotinia sclerotiorum* infects a large number of herbaceous and woody ornamentals, including monocots and dicots, angiosperms and gymnosperms, and one species of fern (Boland and Hall 1994; Farr and Rossman 2017). Gleason et al. (2009) listed over 30 genera of herbaceous perennials and

Daughtrey et al. (1995) reported 10 genera of common flowering potted plants that are susceptible to *S. sclerotiorum*. Boland and Hall (1994) list over 50 susceptible genera in the Asteraceae alone. These lists are outdated as new publications describe infection of previously unreported host plants by *S. sclerotiorum* (Chang et al. 1997; Garibaldi et al. 2008a,b,c; Garibaldi et al. 2001; Grabowski and Malvick 2015, 2017; Gulya et al. 2006; Strauss and Dillard 2009).

Little information is available about white mold in ornamental plants. Many ornamentals appear on published disease indices from various locations and do not include a description of symptoms or severity of the infection (Boland and Hall 1994; Farr and Rossman 2017). A few reports provide more detailed information including symptoms, plant parts infected, and environmental conditions in which disease is likely to occur (Gulya et al. 2006; Jones and Benson 2001; White 1993). Where descriptions are available, symptoms vary between host plants.

In flowering woody ornamentals, descriptions of disease indicate that ascospores initiate infection. In Camilla and forsythia, water soaked lesions form on flower petals in cool wet weather; conditions that are favorable for caropgenic germination. Infections on Camilla are limited to blossoms, but in forsythia infections progress into branch tissue resulting in girdling cankers and wilt of individual branches (Jones and Benson 2001).

In herbaceous ornamentals, evidence of both ascospore and hyphal infection exists. Many reports describe necrotic lesions that start on the lower stem or crown, followed by wilt and death of plants (Daughtrey et al. 1995; Gleason et al. 2009). In production of geranium transplants, mycelia has been observed growing across the soil surface to infect neighboring plants (White 1993). Root and crown rot were observed in

field grown coneflowers (Chang et al. 1997), but Gulya et al. (2006) were unable to initiate infection in *Cuphea* through inoculation of roots. These results indicate that infection at and below the soil line can occur in herbaceous ornamentals but that the ability of *S. sclerotiorum* to infect roots varies by host. It is unclear if basal infections on herbaceous ornamentals are initiated through myceliogenic germination of sclerotia as seen in sunflower or through ascospore colonization of wounded or senescent leaf tissue as seen in lettuce. Infection of flowers, leaves, and aerial portions of the stem has been observed in geranium and cuphea (Gulya et al. 2006; White 1993). This indicates that ascospore infection can occur in some herbaceous ornamentals.

The majority of reports of white mold on ornamentals originate from commercial production of plants in greenhouses or nurseries. (Daughtrey et al. 1995; Garibaldi et al. 2008a,b,c; Garibaldi et al. 2001; Jones and Benson 2001; White 1993). As a result, little is known about the epidemiology of white mold in ornamental plants grown in landscapes.

Disease management. Management of disease caused by *S. sclerotiorum* is challenging and varies by crop. Due to the longevity of sclerotia in soil, rotation to non-susceptible crops provides little reduction in disease severity (Schwartz and Steadman 1978) for most crops. Cultural control practices such as reduced irrigation as well increasing space between plants and rows is recommended to facilitate drying of foliage and flowers and reduce conditions favorable to caropgenic germination and ascospore infection (Grau and Radke 1984). This management strategy becomes ineffective if environmental conditions highly favorable to infection occur. In crops like soybean, canola, and potato where infection of flowers via ascospores plays a critical role in

infection, fungicide sprays timed to protect blossoms are often utilized to reduce disease incidence (Atallah and Johnson 2004; Bradley et al. 2006). Timing of applications is critical and sprays can be costly.

Host resistance. Host resistance is highly sought after as a means to effectively and economically manage white mold. Large collections of varieties and wild relatives of susceptible crops have been evaluated for resistance to *S. sclerotiorum* (Hoffman et al. 2002; Jensen et al. 2008; Porter et al. 2009; Sackston 1992; Sedun et al. 1989). In all cases, the pathogen was able to infect every line tested. Reduced disease severity was observed in some lines, but no immunity or high levels of resistance were found.

In order to better understand the host genetics responsible for partial resistance, breeders conducted quantitative trait loci (QTL) analysis of soybean, dry bean, sunflower, and canola (Bert et al. 2004; Castaño et al. 1993; Kim and Diers 2000; Maxwell et al. 2007; Mestries et al. 1998; Miklas 2007; Wei et al. 2014; Zhao and Meng 2003; Zhao et al. 2007). These studies demonstrated the presence of multiple QTL associate with reduced disease severity and confirmed the polygenic nature of the observed partial resistance. QTL studies also revealed that genes at different loci confer resistance in seedlings versus mature plants (Zhao et al. 2007), in leaves versus flowers (Mestries et al. 1998), to ascospore infection versus mycelial growth (Mestries et al. 1998), and to escape mechanisms like timing of flowering and plant height versus genetic resistance (Kim and Diers 2000). QTL have been utilized to aid in the integration of resistant genes into susceptible varieties (Kim and Diers 2000; Miklas 2007). In the absence of high levels of resistance, breeders strive to integrate multiple forms of partial resistance into agronomically viable varieties.

Escape mechanisms. Reduced incidence and severity of white mold has been observed due to escape mechanisms such as changes in timing and duration of flowering and modification of plant morphology. Dry bean varieties with an open upright growth form, exhibit reduced incidence and severity due to changes in the canopy microclimate that are unfavorable to ascospore infection and mycelial growth (Blad et al. 1978; Park 1993; Weiss et al. 1980). Delayed flowering in beans and canola reduces disease incidence because flowers are produced when environmental conditions are less favorable to infection (Kim and Diers 2000; Wei et al. 2014). Pedraza et al. (2004) demonstrated that sunflowers with moderate resistance to head blight had a reduced window of susceptibility due to a shorter period of time in which anthers, the infection court for sunflower head rot, were present within the head. In peas, increased stem thickness and shorter distances between nodes have been correlated with reduced disease severity, although the mechanisms behind this resistance are not well understood (Porter et al. 2009).

Host defense mechanisms. In addition to escape mechanisms, partial resistance to *S. sclerotiorum* can be conferred by numerous plant defense mechanisms including structural and chemical changes to cell walls, production of phytoalexins, pathogenesis related proteins (PR), cell wall degrading enzymes, and proteins associated with the oxidative burst (Dai et al. 2006; Hegedus et al. 2008; Zhao et al. 2007). Studies of *Brassica napus* demonstrate that the salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signal transduction pathways are induced in response to infection with *S. sclerotiorum* and result in upregulation of resistance genes (Hegedus et al. 2008; Wang et al. 2012; Zhao et al. 2007).

Lignin and phenols are defensive compounds that may be present in plant cells prior to infection or accumulate in response to pathogen attack. Lignin has been shown to resist cell wall degrading enzymes of several plant pathogens (Ride 1978). Resistance to *S. sclerotiorum* in sunflower occurs due to the presence of lignified sclerenchyma and cortical fiber cells that restrict the spread of *S. sclerotiorum* (Mondolot-Cosson and Andary 1994) as well as by post infection accumulation of lignin in vessel and parenchyma cells (Eynck et al. 2012). Phenols are often antimicrobial and have been observed to accumulate in host cell walls in response to infection (Nicholson and Hammerschmidt 1992). Production of the phenols, ayapin, scopoletin, and isochlorogenic acid all increased in the tissue of resistant sunflower lines in response to infection and were shown to inhibit mycleial growth of *S. sclerotiorum* (Bazzalo et al. 1985; Prats et al. 2003; Urdangarín et al. 1999).

Pathogenesis-related proteins (PRs), cell wall degrading enzymes, and other defensive proteins are expressed in response to infection by *S. sclerotiorum* in many host plants (Jung et al. 1993; Jung et al. 1995; Zhao et al. 2007; Dai et al. 2006; Hegedus et al. 2008). Dai et al. (2006) demonstrated that production of chitinase and β, 1-3 glucanase in *Arabidopsis* infected by *S. sclerotiorum* increased as pH of plant tissue decreased, indicating host recognition and response to modification of the apoplast by the pathogen. *Brassica napus* upregulated production of multiple polygalacturanase inhibitor proteins in response to infection by *S. sclerotiorum* (Hegedus et al. 2008). These proteins directly target and disable cell wall degrading enzymes produced by the pathogen. Defense related proteins are often expressed at higher levels in plants with partial resistance (Zhao

et al. 2007) and reduced disease severity is observed in plants where production of defensive proteins is stimulated prior to infection (Fernando et al. 2007).

In acknowledgement of the critical role that oxalic acid plays in pathogenicity, breeders have genetically modified susceptible hosts with oxalate oxidase (*oxo*) or oxalate decarboxylase (*oxdc*) genes. These enzymes break down oxalic acid into nontoxic compounds. Expression of OA degrading enzymes has been observed in host cell walls surrounding the site of infection in transformed plants (Donaldson et al. 2001). Reduction of disease severity has been observed in susceptible crops transformed with *oxo* (Cober et al. 2003; Donaldson et al. 2001; Hu et al. 2003) and *oxdc* (Cunha et al. 2010; Dias et al. 2006; Kesarwani et al. 2000), but the level of resistance varied greatly. Dias et al. (2006) identified several symptom free lettuce lines transformed with *oxdc* in detached leaf assays. In contrast, soybean lines modified to express *oxo* had reduced disease severity compared to unmodified susceptible lines but transgene expression did not provide greater disease control than existing resistant varieties (Cober et al. 2003).

Summary. Although much is known about the biology of *S. sclerotiorum* and the epidemiology of white mold in a few economically important crops, the variable characteristics of this disease among crops limits the ability to successfully apply known information to less well studied plants. Although reports of white mold in ornamental landscape beds are common, little is known about the epidemiology or management options in these production systems. The objectives of this study were to (i) evaluate commonly planted herbaceous ornamental species with no reported history of white mold for resistance to *S. sclerotiorum* in field and controlled environments, (ii) to determine

the effect of temperature and wounding on disease severity, (iii) to compare infection and penetration of *S. sclerotiorum* in select plant species, and (iv) to determine if oxalate oxidase is produced as a defense response to *S. sclerotiorum* in ornamental grasses. Identification of resistant ornamental plants would provide landscape managers with an economical management option for landscape beds infested with *S. sclerotiorum*. In addition, this research furthers the understanding of the host range of *S. sclerotiorum* and suggests potential new sources of genetic resistance that could be incorporated into susceptible crops.

Chapter 2

Evaluation of annual bedding plants for resistance to white mold

Synopsis. White mold, caused by the necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, causes stem rot, crown rot, wilt and death of many common herbaceous ornamental plants. Relatively little research has been done on management of white mold and resistance to this disease in ornamental crops. Plant varieties from four genera of widely available annual bedding plants with no reported history of white mold susceptibility were evaluated for potential resistance to *S. sclerotiorum.* All plants were inoculated with three isolates of *S. sclerotiorum* and evaluated for disease severity in a controlled environment and under field conditions. *Portulaca grandiflora* Hook. 'Sundial Scarlet', *P. grandiflora* 'Sundial White', *Pentas lanceolata* Forssk. "Graffiti Pink', and *Scaevola aemula* R. Br. 'Whirlwind White' were highly susceptible to white mold in the controlled environment, but had significantly lower disease incidence and severity than *Zinnia elegans x angustifolia* 'Profusion White', the susceptible control, under field conditions. *Impatiens hawkeri* W. Bull. 'Sonic Red', *I. hawkeri* 'Sonic Amethyst', *I. hawkeri* 'Sonic White', *I. walleriana* Hook. f. 'Blitz 3000 Red', *I. walleriana*'Blitz 3000 White' and *I. walleriana* 'Blitz 3000 Rose' displayed abscission of diseased plant tissue as an unusual resistance response. Plants from all four genera that were evaluated became infected with *S. sclerotiorum* to a lesser extent than susceptible controls under field conditions and could be used as part of an integrated disease management program for white mold in ornamental plantings.

Introduction

White mold, also called Sclerotinia stem rot, is caused by the fungus *Sclerotinia sclerotiorum*. This necrotrophic pathogen can infect over 400 species of plants from 75 families, including monocotyledons and dicotyledons and herbaceous and woody ornamentals (Boland and Hall, 1994). Gleason et al. (2009) listed over 30 genera of herbaceous perennials and Daughtrey et al. (1995) reported 10 genera of common flowering potted plants that are susceptible to *S. sclerotiorum*. Boland and Hall (1994) list over 50 susceptible genera in the Asteraceae alone. These lists are outdated as multiple new publications describe infection of new host plants by *S. sclerotiorum* (Chang et al., 1997; Garibaldi et al., 2001, 2008a, 2008b, 2008c; Gulya et al., 2006; Strauss and Dillard 2009).

Annual bedding plants are widely used and highly valued in landscapes in private homes, public parks and around commercial properties. The United States Department of Agriculture-Agricultural Statistics Service reports that the value for wholesale annual bedding and garden plants totaled $1.36 billion in 2012.
Many of the most popular annual bedding plants are susceptible to white mold including petunia, zinnia, verbena, snapdragon, and salvia (Boland and Hall, 1994). Infection of annual bedding plants can cause significant plant death in ornamental beds in home and public landscapes. Although infection may start early in the season, plant death from white mold often occurs midsummer, when replacement plants are unavailable.

Infestation of an annual flower bed with *S. sclerotiorum* is particularly problematic because the fungus produces long-term resting structures called sclerotia (Bolton et al., 2006; Grau and Radke, 1984). Once introduced, the fungus can persist in

soil and plant debris for decades with repeated infection each year. The close plant spacing and overhead irrigation used in most annual plantings create highly favorable environmental conditions for sporulation and infection by *S. sclerotiorum* (Bolton et al., 2006; Grau and Radke, 1984).

Although white mold causes significant levels of stem rot, crown rot, wilt and death of many common herbaceous ornamental plants (Bolton et al., 2006), relatively little research has been done on management of white mold in ornamental crops. Annual bedding plants are not commonly evaluated for resistance to white mold. There are several commonly used genera and species of annual bedding plants for which susceptibility or resistance to white mold has not been reported. Identification of genera or species with resistance to *S. sclerotiorum* would provide an effective means of managing white mold, requiring little extra labor or expenses. The objective of this study was to evaluate widely available annual bedding plant varieties with no reported susceptibility to white mold for potential resistance to this disease.

Materials and Methods

***Isolate preparation*.** Three isolates of *S. sclerotiorum* from diverse hosts were arbitrarily chosen for this study. The isolates were ORN010, isolated from a petunia plant in Chaska, MN; FC002, isolated from a soybean plant in Renville County, MN; and VEG001, isolated from green bean in Spring Valley, WI. All isolates were grown on autoclaved carrots and stored as sclerotia. Sclerotia were later germinated on half strength potato dextrose agar (PDA) (Difco, Sparks, MD) for use in inoculation trials. Isolates

were never transferred on PDA more than three times between plant inoculations in order to maintain pathogenicity.

***Studies of disease susceptibility in controlled environments*.** Plant species and varieties for these studies were chosen based on the known host list of *S. sclerotiorum* (Boland and Hall, 1994; Farr et al., 1989), the 2009 University of Minnesota annual flower trial list, and several commercial flower catalogues. Plant families were identified that had no known hosts to *S. sclerotiorum,* and genera of annual bedding plants within these families were chosen that are widely available to landscape managers (Table 1). The annual bedding plants were purchased in March as transplants from a local garden center in 4 inch pots or 9 packs. The plants in 9 packs were transplanted into 4 inch pots and grown in a greenhouse with 12 h light at 25 ^{0}C and 12 h darkness at 20 ^{0}C for two weeks prior to inoculation.

Studies in growth chambers were arranged in a completely random design with two factors; isolate and plant entry, replicated three times. One primary stem per plant was cut with a sterile razor blade just below the upper- most pair of fully expanded leaves. An 8 mm square of half strength PDA containing the leading edge from a *S. sclerotiorum* culture was placed on the tip of the cut stem and covered by a sterile 0.6 ml microcentrifuge tube. One uninoculated control plant per plant entry was prepared for inoculation in a similar fashion but received only sterile half strength PDA. *Zinnia elegans x angustifolia* 'Profusion White' was included as a known susceptible control.

All plants were incubated in a mist chamber with a 12 h photoperiod and misted 10 minutes every hour for two days after inoculation. After two days, plants were moved to a growth chamber (Environmental Growth Chambers, Chagrin Falls, OH, U.S.A.) set

to 18 ^{0}C for 14 h of light and 16 ^{0}C for 10 h of darkness. Lesion length was measured at 4 and 14 days after inoculation (DAI) as a rating of disease severity. Due to a unique and unexpected resistance response of abscission of infected stems, lesion length could not be accurately measured for impatiens and New Guinea impatiens. The following scale was used to rate all impatiens: 1 – stem abscission halted progression of disease, 0 – stem abscission did not halt progression of disease. At 14 DAI, a 2.5 cm section of the inoculated stem centered on the leading edge of visible symptoms was excised, surface sterilized and placed on half strength PDA to verify that *S. sclerotiorum* was the causal agent of the observed symptoms. The experiment was performed twice.

Field studies of disease susceptibility. All plant entries were transplanted in a randomized complete block design with six blocks at two locations in 2011 on the St. Paul campus of the University of Minnesota. Plants were purchased in 4 inch pots or 9 packs from a local garden center and transplanted into one field plot on 8 June and the other field plot on 14 June. Each entry was transplanted in a 92 cm x 92 cm bed with 305 cm between plants. A single row of 'Profusion Cherry' zinnias was planted as a border around each block to help maintain even moisture within the beds and reduce edge effects. Plants were watered once a day for 20 minutes with an oscillating sprinkler. Osmocote 14-14-14 was applied 2 weeks after planting at a rate of 340 kg/ha to all plots.

The three isolates of *S. sclerotiorum* noted above were grown on half strength PDA. When cultures reached the edge of a 100 mm Petri plate, the agar from three plates per isolate was cut into 10 mm squares using a sterile spatula and placed into a sterile 48 x 58 cm autoclavable bag (VRW Intl. Radnor, PA, U.S.A.) containing 2000 cc of sorghum seed. Prior to inoculation sorghum seed was soaked in distilled water for 18 to

24 h, strained to remove excess water, autoclaved for 60 min, allowed to cool for 18 to 24 h, and autoclaved a second time for 60 min. The fungus was allowed to colonize the seed for three weeks at 23 ^{0}C and then the infested seed was dried at 35 ^{0}C for 48 h and ground to a course powder in a standard blender.

As no affect due to isolate was identified in the controlled environment experiments, all three isolates were combined in field studies. Inoculum was created by mixing 1,000 cc of ground seed from each of the three isolates. The inoculum mixture was then sprinkled evenly over the plant canopy by hand at a rate of 86.5 l/ha on August 4th 2011 when the trial plants had grown together to achieve canopy closure and again one week later. Immediately after inoculation, plants were irrigated for five minutes. Irrigation was subsequently applied three times daily for 10 minutes for the duration of the experiment. Following the inoculations, the daily maximum temperature ranged from 22.7 ^{0}C to 29 ^{0}C and the daily minimum temperature ranged from 15.5 ^{0}C to 23 ^{0}C.

Disease severity and incidence were recorded every 14 days after inoculation until frost. Disease incidence was recorded as the number of plants per bed that were visibly infected with *S. sclerotiorum* based on signs and symptoms. Any plant with questionable symptoms was surface sterilized and plated on half strength PDA at the end of the experiment to isolate and check for infection by *S. sclerotiorum*. Disease severity was recorded as percent canopy missing due to death of plants within the nine plant beds. In order to compare disease progress over time, the summary variable area under disease progress curve (AUDPC) (Madden et al., 2007) was calculated for a 28 day period for field 2 and a 42 day period for field 1. This difference in period of observation for the two fields was the result of delayed planting and an early frost in field 2.

All varieties of *I. walleriana* were excluded from the final analysis of disease severity and incidence due to the confounding effects of severe infection by *Plasmopara obducens*, the causal organism of Impatiens downy mildew. *Pentas lanceolata* Graffiti Bright Red® was not included in the field trial due to a crop failure at the nursery.

***Statistical analysis*.** Using Hartley's Fmax test, it was determined that variances were homogeneous between both controlled environment experiments. As a result, data from both experiments were combined and analyzed as a factorial design using JMP software to calculate an ANOVA. Tukey's Honestly Significant Difference was used to separate means. Uninoculated controls were not included in statistical analysis but were maintained for comparison.

Data from field experiments was not normal and treatment variance was not equal. This could not be corrected with standard data transformations. As a result, non-parametric methods were used to analyze the data. Friedman's test was used to calculate a chi-squared 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 differences between treatments.

Results

***Studies in controlled environments*.** All inoculated plants became infected with *S. sclerotiorum.* The pathogen was successfully isolated from all symptomatic tissue. Disease severity varied among the plants tested (Table 2). *Scaevola aemula* 'Whirlwind White' had significantly greater disease severity than all other plants tested except the

susceptible control ‘Profusion White’ zinnia. Disease severity of the *Portulaca grandiflora* and *Pentas lanceolata* varieties was significantly less than the susceptible control four days after inoculation, but by day 14 there was no significant difference between disease severity in these varieties and the susceptible control (Table 2). By day 14 of the experiment, 100% of the susceptible control plants, ‘Profusion White’ zinnia, were infected and lesion length extended the entire length of the stem. *S. sclerotiorum* was recovered from the leading edge of all lesions tested. There was no significant difference in disease severity caused by the three isolates of *S. sclerotiorum*. In addition, there were no significant interactions between isolate and plant entry (data not shown).

All varieties of *I. hawkeri* and *I. walleriana* developed a unique abscission resistance response to infection with *S. sclerotiorum*. Initially, the inoculated stem developed soft rot at the point of inoculation, and occasionally blue-black steaks developed along the stem beyond the rotted area. This was most easily observed on varieties with green stems. At healthy nodes in advance of the infection the plant created an abscission zone in the stem, and the stem above the abscission zone completely detached from the lower stem as shown in Fig. 1. Nearly 100% of all three varieties of *I. hawkeri* completely recovered from infection through this resistance response (Table 3). All varieties of *I. walleriana* tested also developed an abscission layer, but *S. sclerotiorum* frequently infected the lower stem before complete abscission occurred and soft rot often progressed to the crown of the plant (Table 3). *S. sclerotiorum* was recovered from areas of the impatiens stem with clear soft rot symptoms both above and below the abscission layer. If the plant successfully abscised the stem section containing the infection, *S. sclerotiorum* was not recovered from the stem below the abscission layer.

Studies under field conditions. All plant entries tested under field conditions developed infection by *S. sclerotiorum*, but incidence and severity varied (Table 4). In contrast to observations in controlled environment conditions, all varieties tested had lower disease incidence and severity compared to the susceptible control including *S. aemula*. No consistent difference between plant entries was observed in disease incidence or severity. The abscission resistance response observed in *Impatiens* spp. under controlled environment conditions was also observed in both species in field conditions.

Discussion

This is a first report of susceptibility of *I. hawkeri*, *I. walleriana*, *Pentas lanceolata*, *Portulaca grandiflora* and *Scaevola aemula* to white mold caused by *S. sclerotiorum*. This study shows that *S. aemula*, *P. grandiflora*, *Pentas lanceolata* and *Impatiens* spp. are not immune to infection by *S. sclerotiorum* but have some resistance to infection under field conditions and suffer less canopy dieback than the susceptible control. As a result, landscape managers could use these plants as part of an integrated disease management plan to reduce damage from white mold. In addition, these results indicate that trials conducted in controlled environmental conditions alone are not sufficient to evaluate plant species or varieties for susceptibility to white mold.

The stem abscission observed in *Impatiens* spp. in response to infection with *S. sclerotiorum* was unexpected, unique and very effective in preventing disease development. Only one other report of abscission as a defense response to *S. sclerotiorum* was found. Sedun et al. (1989) reported that abscission of infected leaf

petioles of *Brassica carinata* in response to infection by *S. sclerotiorum* prevented the infection from spreading to the main stem. This defense response was not observed in any other *Brassica* sp. tested. Ethylene and jasmonic acid are known signal molecules for disease defense and abscission in plants (Dostal et al., 1991; Estornell et al., 2013; González-Carranza et al., 1998; Sequeira, 1973; Sexton, 1976; Wilson et al., 1987), and it may be beneficial to further study the role that plant hormones may play in the resistance response between *Impatiens* spp. and *S. sclerotiorum*.

Varieties of *Scaevola aemula*, *Portulaca grandiflora* and *Pentas lanceolata* demonstrated higher levels of resistance under field conditions than in a controlled environment growth chamber. In a similar study comparing greenhouse and field inoculations of soybean, sunflower and dry bean, Vuong et al. (2004) demonstrated a correlation between disease severity in greenhouse and field evaluations of dry bean varieties, but found no correlation in sunflower and varying results for soybean. In studies evaluating field and vegetable crops, genetic resistance is often attributed to varieties that have slower lesion length development (Blanchette and Auld, 1978; Sedun et al., 1989). Varieties of *Scaevola aemula, Portulaca grandiflora* and *Pentas lanceolata* had lesion lengths equal to susceptible control plants fourteen days after inoculation, indicating a lack of genetic resistance to *S. sclerotiorum*. Yet all of these plants had significantly lower disease incidence and severity than the susceptible control under field conditions throughout the growing season.

This field resistance may be due to plant architecture that creates an unfavorable environment for *S. sclerotiorum* in lieu of genetic defense mechanisms. Reduction of white mold disease severity due to plant architecture of bean has been observed in several

studies (Blad et al., 1978; Park, 1993; Weiss et al., 1980). Blad et al. (1978) demonstrated that a dry bean variety with a 'dense luxuriant canopy' had greater leaf wetness, cooler canopy temperature, and greater disease severity than a variety with an 'open, upright growth form' with similar levels of genetic resistance. Park (1993) demonstrated that upright bean varieties had lower disease incidence than short bush types. An increase in leaf area and canopy cover of the soil was also associated with increased disease severity in bean crops (Blad et al., 1978; Weiss et al., 1980).

Scaevola aemula and *Portulaca grandiflora* both have narrow leaves that develop along recumbent stems. The overall plant canopy is open, and each individual stem receives sunlight and air movement. Soil was easily observed in beds below both plant entries in field plots indicating little shading of the soil surface. *Pentas lanceolata* has a tall, open upright plant form with narrow leaves and multiple branches arising from a primary stem. In contrast the susceptible control 'Profusion white' zinnia has a short, dense, compact canopy with multiple stems grouped close together. The growth forms of *Scaevola aemula*, *Portulaca grandiflora* and *Pentas lanceolata* could have accelerated the drying of stems, foliage and soil after irrigation, rain or dew, creating environmental conditions less favorable for infection by *S. sclerotiorum* and development of white mold.

This study reveals that various levels of resistance to white mold exist in readily available but understudied annual bedding plants. Resistance could come in the form of genetic resistance or as physical properties that make the plant a poor host. Evaluation of plants for resistance to white mold should therefore include studies under field conditions as well as in controlled environmental conditions.

Table 1: Plants species and varieties evaluated for susceptibility to white mold.

Scientific name	Variety	Family
Impatiens hawkeri	New Guinea Impatiens Sonic® Red	Balsaminaceae
Impatiens hawkeri	New Guinea Impatiens Sonic™ Amethyst ’06	Balsaminaceae
Impatiens hawkeri	New Guinea Impatiens Sonic™ White	Balsaminaceae
Impatiens walleriana	Impatiens Blitz 3000™ Red	Balsaminaceae
Impatiens walleriana	Impatiens Blitz 3000™ Rose	Balsaminaceae
Impatiens walleriana	Impatiens Blitz 3000™ White	Balsaminaceae
Pentas lanceolata	Graffiti Bright Red®	Rubiaceae
Pentas lanceolata	Graffiti Pink®	Rubiaceae
Portulaca grandiflora	Moss Rose Sundial™ Scarlet	Portulacaceae
Portulaca grandiflora	Moss Rose Sundial™ White	Portulacaceae
Scaevola aemula	Whirlwind® White	Goodeniaceae
Zinnia elegans x angustifolia	Profusion White	Asteraceae

Table 2: White mold lesion length ratings for annual bedding plants inoculated under controlled environment conditions.

Scientific name	Variety	Day 4 Lesion Length (cm)	Day 14 Lesion Length (cm)
Scaevola aemula	Whirlwind® White	7.45 a**	12.72 a
Zinnia elegans x angustifolia	Profusion White	5.99 b	9.68 ab
Portulaca grandiflora	Moss Rose Sundial™ White	3.74 c	6.71 bc
Portulaca grandiflora	Moss Rose Sundial™ Scarlet	3.54 c	7.87 bc
Pentas lanceolata	Graffiti Pink®	2.63 cd	6.53 c
Pentas lanceolata	Graffiti Bright Red®	2.22 d	7.86 bc

**measurements with the same letter are not significantly different at $\alpha = 0.01$

Table 3: Percent of impatiens plants that recovered from infection by *S. sclerotiorum* due to abscission of infected stems and the average abscission rating[z] for each variety.

Scientific name	Variety	Percent plants recovered from infection	Abscission rating 14 DAI
Impatiens hawkeri	New Guinea Impatiens Sonic™ White	100.0	1.00 a**
Impatiens hawkeri	New Guinea Impatiens Sonic® Red	94.4	0.94 a
Impatiens hawkeri	New Guinea Impatiens Sonic™ Amethyst '06	94.4	0.94 a
Impatiens walleriana	Impatiens Blitz 3000™ Rose	72.2	0.72 ab
Impatiens walleriana	Impatiens Blitz 3000™ White	72.2	0.72 ab
Impatiens walleriana	Impatiens Blitz 3000™ Red	50.0	0.50 b

[z] (1 – stem abscission halted progression of disease, 0 – stem abscission did not halt progression of disease)

**measurements with the same letter are not significantly different at $\alpha = 0.01$

Table 4: Area under disease progress curve (AUDPC) for incidence and severity[z] of white mold in field-inoculated annual bedding plants.

		Incidence AUDPC		Severity AUDPC	
Scientific name	**Variety**	**Field 1**	**Field 2**	**Field 1**	**Field 2**
Portulaca grandiflora	Moss Rose Sundial™ Scarlet	13 c**	0 b	1 d	0 b
Portulaca grandiflora	Moss Rose Sundial™ White	358 c	26 b	167 c	0 b
Impatiens hawkeri	New Guinea Impatiens Sonic™ Amethyst ’06	13 c	0 b	0 d	0 b
Impatiens hawkeri	New Guinea Impatiens Sonic® Red	79 c	0 b	33 d	0 b
Impatiens hawkeri	New Guinea Impatiens Sonic™ White	52 c	0 b	0 d	0 b
Pentas lanceolata	Graffiti Pink®	700 b	93 b	109 c	19 b
Scaevola hybrid	Whirlwind® White	1184 b	39 b	657 b	6 b
Zinnia elegans x angustifolia	Profusion White	3163 a	2087 a	1268 a	1048 a

[z]Due to delayed planting and early frost in field 2, AUDPC was calculated over a 28 day period, whereas AUDPC for field 1 was calculated over a 42 day period.

**measurements with the same letter are not significantly different at $\alpha = 0.01$


goldfisch
Sonic® Red
New Guinea Impatiens

Figure 1: Abscission of an *Impatiens hawkeri* stem infected with *Sclerotinia sclerotiorum* prevented progression of the infection further down the stem.

Chapter 3

Evaluation of ornamental tropical plants for resistance to white mold caused by *Sclerotinia sclerotiorum*

Synopsis. *Sclerotinia sclerotiorum* (Lib.) de Bary is a fungal pathogen that causes stem rot, crown rot, wilt, and death of many common annual flowering plants. Infested flower beds often suffer significant plant loss each year, and identification of disease resistant plants would be a useful management tool. Caladium (*Caladium ×hortulanum* Birdsey), canna *(Canna ×generalis* L.H. Bailey), and elephant ear (*Colocasia esculenta* (L.) Schott) have no reported susceptibility to *S. sclerotiorum* and were evaluated for potential resistance in this study. Plants grown in field conditions in Minnesota in 2012 and 2013 were inoculated through application of sorghum grains colonized by *S. sclerotiorum*. The number of plants infected and percent of canopy dieback was recorded weekly for 3 months. The susceptibility of leaves, flowers, and below ground storage organs also was examined through direct inoculation of plant tissue with a mycelial plug of the pathogen in controlled environmental conditions favorable for disease development. Symptoms and progression of the infection were recorded after 24 days. Symptoms of infection on were similar in field and controlled environments. Caladium plants were susceptible to *S. sclerotiorum*. Petioles, leaves, and corms developed a watery soft rot. Elephant ear was highly resistant to infection. *Sclerotinia sclerotiorum* infected only wounded or senescent tissue and did not result in significant symptoms under any conditions. Canna had partial resistance to the pathogen. Although canna flower petals were readily infected, infection of petioles was restricted to small necrotic lesions. Neither infection progressed to the main stem or resulted in plant death. This study indicates that canna and elephant ear have resistance to *S. sclerotiorum* and could be utilized in an integrated disease management program for infested landscape beds.

Introduction

White mold, caused by the necrotrophic fungal pathogen *Sclerotinia sclerotiorum*, causes stem rot, crown rot, wilt, and death of many common annual bedding plants including zinnia, petunia, verbena, snap dragon, and salvia (Boland and Hall, 1994; Farr and Rossman, 2017; Grabowski and Malvick, 2015). Infested flower beds often suffer significant plant loss each year due in part to the ability of the pathogen to survive for at least 8 years in soil (Bolton et al., 2006). Infection occurs in cool moist conditions and plant death from white mold occurs in mid- to late summer (Abawi and Grogan, 1979; Bolton et al., 2006). At this stage of the growing season, replacement plants for summer flowering annuals are typically no longer available. Garden managers are left with unsightly patches of dead plants or bare soil.

Identifying ornamental plants with resistance to white mold would allow growers to avoid disease problems in beds known to be infested with *S. sclerotiorum*. Over 400 plant species from more than 75 families are known to be susceptible to *S. sclerotiorum* (Boland and Hall, 1994). Gleason et al. (2009) list over 30 genera of herbaceous perennials and Daughtrey et al. (1995) list 10 common flowering potted plants that are susceptible to *S. sclerotiorum*. New susceptible host plants are regularly identified (Chang et al., 1997; Garibaldi et al., 2008a, 2008b, 2008c, 2001; Grabowski and Malvick, 2015; Gulya et al., 2006; Strauss and Dillard, 2009). Grabowski and Malvick (2015) tested plants in four genera of annual bedding plants with no reported susceptibility to white mold for potential resistance to *S. sclerotiorum*. Although moderate resistance was found, plants from all four genera became infected by *S. sclerotiorum* to some degree. To

identify plants with reliable resistance to white mold, new taxa of ornamental plants need to be evaluated for resistance.

Ornamental tropical plants are often grown in annual flower beds to add color and texture. Canna, caladium, and elephant ear have a long history of cultivation. Canna originates from South America and was first introduced to European gardeners as a foliage plant in 1595 (Khoshoo and Mukherjee, 1970; Prince, 2011). Caladium originates from tropical regions of Central and South America (Germplasm Resources Information Network, 2017). Breeding of hybrid garden caladium has occurred for over 150 years (Deng et al., 2007). Elephant ear, also known as taro, originates from Southeast Asia and is believed to be one of the oldest domesticated crops (Quero-Garcia et al., 2010). Plants from these genera are readily available to landscape managers in a variety of colors to meet designers' needs. Cultivation and care of these plants in gardens is well described (Iversen, 1999), allowing for easy adoption of plants should they prove resistant to white mold.

Perhaps because they are of tropical origin, plants in these genera have few pest problems in temperate climates (Horst, 2001; Iversen, 1999) and no recorded history of infection by *S. sclerotiorum* (Boland and Hall, 1994; Farr and Rossman, 2017). In addition, all are monocots. Although some monocots are listed as hosts to *S. sclerotiorum*, economic damage due to this pathogen primarily occurs in dicotyledonous plants (Boland and Hall, 1994; Bolton et al., 2006; Farr and Rossman, 2017).

Although canna, caladium, and elephant ear are grown as annuals in temperate climates, each produces a below ground storage organ (rhizome, tuber, corm) that can be dug up at the end of the season, stored for the winter, and planted the following season.

Below ground storage organs can be used to propagate these plants as well (Iversen, 1999). In addition to evaluating resistance to infection of above ground plant parts, it is important to determine the susceptibility of corms, tubers, and rhizomes. White mold is known to spread in storage on crops like carrot (Koike, 2007). If corms, tubers, and rhizomes of tropical ornamentals were infected, similar spread in storage would be likely.

The objective of this study was to evaluate three commonly grown tropical ornamentals, canna, caladium, and elephant ear, for potential resistance to *S. sclerotiorum*.

Materials and Methods

***Plant materials for field studies*.** All plants were grown in composted bark potting mix (Sungro Horticulture, Agawam, MA) with one plant per pot in a greenhouse with 12 h light at 25 ^{0}C and 12 h darkness at 20 ^{0}C prior to transplanting into field plots. Cultivars that are readily available to garden managers were chosen for the experiment. Corms of 'Ruffles' elephant ear, purchased from a local garden center, were grown in 25.4 cm round pots for 10 weeks. Tubers of 'White Queen' caladium that were 3.8 to 6.4 cm in diameter (Classic Caladiums, Avon Parks, FL) were grown for 5 weeks in 10.2 cm pots. Seeds of 'Tropical White' canna (Stokes Seeds Inc., Buffalo, NY) were planted 12 weeks prior to the start of the experiment in 10.2 cm pots. *Zinnia elegans* × *angusifolia* 'Profusion White' seedlings, included as a known susceptible control, were purchased from a local garden center and transplanted into 10.2 cm pots two weeks prior to the start of the experiment. All plants had 2-5 fully expanded leaves at the time of transplant.

***Inoculum preparation*.** Three isolates of *S. sclerotiorum* were used in the experiment: ORN010, isolated from a petunia plant in Chaska, MN; FC002, isolated from a soybean plant in Renville County, MN; and VEG001, isolated from green bean in Spring Valley, WI. Sclerotia of each isolate were produced and stored as described in Grabowski and Malvick (2015).

Field studies of disease susceptibility. All plant entries were transplanted on June 5 in a randomized complete block design with four replicated blocks on the Minnesota Agricultural Experiment Station, St. Paul campus. The experiment was conducted twice, in 2012 and 2013. Each experimental unit contained four plants of the same cultivar in two rows of two plants. Plants were spaced to create canopy closure at the same time despite differences in plant size. Zinnia and caladium plants were spaced 25.4 cm apart within plots. Canna plants were planted 40.6 cm and 35.6 cm apart in 2012 and 2013, respectively, and elephant ear plants were planted 50.8 cm and 35.6 cm apart in 2012 and in 2013 respectively due to differences in transplant size. Plants were watered once a day for 20 min with an oscillating sprinkler prior to inoculation. Osmocote 14-14-14 was applied 2 weeks after planting at 340 kg/ha to all plots.

Plots were inoculated with a mixture of equal quantities of sorghum seed colonized by three isolates of *S. sclerotiorum*, which was prepared as described by Grabowski and Malvick (2015). The inoculum mixture was spread evenly over the plant canopy at 86.5 L/ha when the trial plants had grown together to achieve canopy closure and again one week later. Canopy closure was reached in the fourth week of July in both years. Irrigation was applied with an oscillating sprinkler for 5 min immediately after inoculation and subsequently applied three times daily for 10 min for the remainder of the

experiment. Following the inoculations, the daily maximum temperature ranged from 24 ^{0}C to 33 ^{0}C in 2012 and 24 ^{0}C to 30 ^{0}C in 2013 and the daily minimum temperature ranged from 14 ^{0}C to 24 ^{0}C in 2012 and 14 ^{0}C to 18 ^{0}C in 2013.

Disease incidence and severity were recorded every 7 days after inoculation until mid-September. Disease incidence was recorded as the number of plants per plot that were visibly infected with *S. sclerotiorum* based on signs and symptoms. Any plant with questionable symptoms was surface disinfested with an aqueous solution of 0.525% sodium hypochlorite and placed on half-strength potato dextrose agar medium (1/2x PDA, Becton, Dickinson and Company, Franklin Lakes, NJ) at the end of the experiment to confirm infection by *S. sclerotiorum*. Disease severity was recorded as percent canopy missing or dead due to infection of plants within the bed. The area under disease progress curve (AUDPC) was calculated to compare disease progress over time (Madden et al., 2007).

***Studies of disease susceptibility in controlled environments*.** Plants were grown for inoculation as described above. 'Fannie Munson' caladium and 'Tropical Yellow' canna were added to the trial. To produce small plants that were suitable for use in growth chamber trials, corms of 'Ruffles' elephant ear were propagated from plants maintained in the greenhouse as described above during March through September one year prior to the experiment. In October, stems were cut off at the soil line and pots were allowed to dry for 2 weeks. The corm and potting soil from all pots was placed in a plastic bag and stored at 10 ^{0}C. Prior to the experiment, potting soil was gently removed from the corms; corm offsets were broken off the central bulb by hand and sorted by size. All offsets were carefully inspected; any offsets showing signs of rot or injury were

discarded. Corm offsets that were 2.5 to 5 cm in diameter were planted 10 weeks prior to the start of the experiment in 12.7 cm square pots. All plants were maintained in a greenhouse as described above and had 2-5 fully expanded leaves prior to inoculation. Sclerotia from each of the three isolates described above were placed individually on 1/2x PDA five days prior to the experiment and incubated at 22 ^{0}C to promote mycelial growth.

Inoculation of foliage. The experiment was a completely random design with two factors, isolate and plant entry, replicated three times with one plant per replication. An 8 mm square of agar containing the leading edge from one of the three isolates of *S. sclerotiorum* culture was placed between the plant stem and the petiole of the oldest leaf of the plant that was still green and healthy. Mock inoculated control plants were prepared for inoculation in a similar fashion but received only sterile 1/2x PDA. All plants were incubated in a mist chamber with a 12 h photoperiod at 22 ^{0}C and misted 10 minutes per hour for 2 days after inoculation. Then plants were moved to a growth chamber (Environmental Growth Chambers, Chagrin Falls, OH) set to 18 ^{0}C for 14 h of light and 16 ^{0}C for 10 h of darkness. Plants were hand watered as needed with water directed to the soil. No significant differences in disease severity were observed on the plants inoculated with the different isolates of *S. sclerotiorum* and no interactions between isolate and plant entries were detected (data not shown). Data from all three isolates was combined for analysis of foliar infection data.

Disease severity was assessed 24 days after inoculation (DAI) as the percent of leaves wilted or killed per plant. Plants were taken out of the pots and soil was carefully removed from the surface of the corm, tuber, or rhizome. Corms, tubers, and rhizomes

were bisected vertically with a sterile knife and the depth of rot was measured from the point of leaf attachment downward toward the roots. A section of plant tissue was cut from the leading edge of the infection, surface disinfested with an aqueous solution of 0.525% sodium hypochlorite, and plated on 1/2x PDA. All plates were examined for the presence of *S. sclerotiorum* after 14 days of incubation at 22 ^{0}C. The experiment was conducted twice.

Inoculation of elephant ear petioles. Natural senescence of the outermost leaf in elephant ear plants confounded results in the foliar inoculation study. To determine if *S. sclerotiorum* can infect non-senescent petioles of 'Ruffles' elephant ear, an 8 mm square of 1/2x PDA containing the leading edge of a culture of isolate ORN010 was placed midway between the base of the petiole and the base of the leaf. Parafilm® (Bemis Company, Inc., Oshkosh, WI) was wrapped around the inoculum and petiole to secure it to the plant. Each plant was inoculated on a newly expanded inner leaf, and the outermost, oldest leaf in the whorl. Mock inoculated controls received a sterile 8 mm square of 1/2x PDA. The experiment was a completely random design with three replications. Plants were incubated as described above and moved to a growth chamber (Environmental Growth Chambers, Chagrin Falls, OH) with a 12 h photoperiod set to 13^{0}C to determine if resistance of the tropical plants persisted at the low end of the pathogen's temperature range for infection (Bolton et al., 2006), which commonly occurs during the growing season in northern states. Symptoms were recorded 28 DAI. All inoculated petioles were surface disinfested with an aqueous solution of 0.525% sodium hypochlorite, and five 1 cm cross sections were placed on 1/2x PDA and examined for

the presence of *S. sclerotiorum* after 14 days incubation at 22 ^{0}C. The experiment was conducted twice.

To determine if wounding played a role in infection of elephant ear petioles, the inoculation was repeated as described above for newly expanded leaves, with the modification of a shallow 1 cm wide wound being created on the petiole with a sterile razor blade at the inoculation site prior to inoculation. The wounding experiment was conducted twice.

Inoculation of canna flowers. Of the three genera, canna is the only plant that will flower regularly in northern gardens. As a result, it was important to determine if *S. sclerotiorum* could infect canna through the inflorescence. Flowers of 'Tropical White' and 'Tropical Yellow' canna were tested in a completely random design with three replications and one plant per replication. An 8 mm square of 1/2x PDA containing the leading edge from a culture of isolate ORN010 was placed at the center of a newly opened flower of an inflorescence with 60-80% open blossoms. Mock inoculated controls received sterile 1/2x PDA. Plants were moved to a growth chamber set to 13^{0}C with a 12 h photoperiod and incubated as described above. Disease severity was rated as the number of flowers infected at 6 and 19 DAI, and as the lesion length on the flower stalks 28 DAI. The trial was conducted twice.

Susceptibility of below ground storage organs in controlled environment. Rhizomes of 'Tropical White' canna were produced by growing plants from seed for 20 weeks in greenhouse conditions as described above. Corms of 'Ruffles' elephant ear were produced through production of offsets as described above. Tubers of 'White Queen' caladium were purchased from a commercial caladium grower (Classic Caladiums, Avon

Parks, FL). Tap roots of food grade carrots, *Daucus carota* subsp. *sativus* (Hoffm.) Schübl. & G. Martens, were included as a susceptible control.

All storage organs were wounded by creating a shallow 1 cm wide wedge with a sterile razor blade. A 1 cm square of 1/2x PDA containing the leading edge from a *S. sclerotiorum* culture, isolate ORN010, was placed directly on the wound. Mock inoculated controls received the same treatment with a sterile square of 1/2x PDA. Following inoculation, the storage organs were placed in a plastic bag, misted with distilled water, and the bags were kept sealed in a growth chamber set to 18 ^{0}C for 14 h of light and 16 ^{0}C for 10 h of darkness to optimize conditions for fungal growth and infection. At 10 DAI, samples were cut in half lengthwise and the extent of discolored and decomposing tissue was recorded based on the length of area that was symptomatic relative to the total length. A 1 cm square sample from the leading edge of the infection was removed, surface disinfested with an aqueous solution of 0.525% sodium hypochlorite, and plated on 1/2x PDA. Samples were incubated for 14 days at 22 ^{0}C and then examined for the presence of *S. sclerotiorum*. The experiment was a completely random design with four replications and one plant per replication. The trial was conducted twice.

Statistical Analysis. Data from controlled environment experiments, direct inoculation of storage organs, and field trials were combined after Hartley's Fmax test demonstrated homogenous variance between repeated trials. Data from controlled environment experiments was analyzed as a factorial design using JMP software (SAS Institute, Inc., Cary, NC). Tukey's honestly significant difference was used to separate means. Data from direct inoculation of storage organs and field trials was not normal and

could not be corrected by standard data transformations; therefore, non-parametric methods were used to analyze the data. Friedman's test was used to calculate a chi-squared value for the model and a p-value for treatments. Treatments with a *P*-value ≤0.05 were compared using Wilcoxon's rank testing to determine differences between treatments.

Results

Studies under field conditions. Disease incidence was 100% at 7 DAI for the susceptible control 'Profusion White' zinnia (Fig. 2). Disease severity (percent canopy killed) in zinnia plots increased in a monomolecular curve until 98% of the plant canopy was killed at 49 DAI (Fig. 3). Average incidence for canna and caladium was $\underline{<}$ 60% throughout the duration of the trials. Incidence for elephant ear was 0% throughout both years (Fig.2). Canopy death in caladium never exceeded 11% and no canopy death was recorded for canna or elephant ear in either year (Fig. 3). The AUDPC for incidence and severity was highest for zinnia followed by caladium, canna, and elephant ear (Table 5).

Studies in controlled environments.

Inoculation of foliage. Inoculated caladium cultivars had significantly greater disease severity than mock-inoculated controls. 'White Queen' caladium had significantly greater disease severity than all other plants tested. Disease severity of elephant ear and canna was not significantly different from mock-inoculated controls (Table 6).

Inoculated caladium petioles developed soft rot, resulting in wilt of one to several leaves as early as 7 DAI. Mycelia and sclerotia were commonly observed on infected petioles at 24 DAI. The infection progressed into the tuber on 44% of 'White Queen' caladium plants and 20% of 'Fannie Munson' caladium plants. Inoculated canna leaves developed dry brown necrotic lesions averaging 3.3 cm in length at the site of infection 24 DAI. This lesion resulted in wilt of the inoculated leaf in 8% of plants. Petiole infection of canna never progressed into the main stem or rhizome. The outermost leaf of both inoculated and mock-inoculated elephant ear plants turned yellow to brown and wilted 24 DAI. *Sclerotinia sclerotiorum* was isolated from inoculated leaves but it was unclear if infection occurred prior to natural leaf senescence. Infection did not progress into elephant ear corms.

Infection of canna flowers. Canna flowers became infected when directly inoculated. Discolored petals and mycelia were observed on petals as early as 6 DAI. Within the inoculated inflorescence, an average of 25% of the flowers were infected 6 DAI and 68% of flowers were infected 19 DAI. By 28 DAI, all flowers had naturally senesced. Flower stalk infection occurred in 100% of plants, averaging 6.5 cm in length, or 48% of the length of the flower stalk. Infected flower stalks developed a brown elongate lesion progressing upward and downward from the infected flower. Infections initiated in the inflorescence never girdled the flower stalk, progressed to the main stem, nor resulted in infection of leaves or secondary inflorescences.

Infection of elephant ear petioles. At 28 DAI, all young newly expanded elephant ear leaves remained green and turgid, and all old leaves from the outer whorl turned yellow and wilted regardless of inoculation with *S. sclerotiorum*. The pathogen

was isolated from 33% of inoculated, old, non-wounded petioles and was not isolated from any inoculated young, non-wounded petioles. Young wounded petioles were asymptomatic at 28 DAI but *S. sclerotiorum* was isolated from 50% of wound-inoculated plants.

Infection of below ground storage organs. Canna rhizomes and elephant ear corms showed no discoloration or rot following inoculation with *S. sclerotiorum* at 10 DAI (Table 7). Tubers (25%) of caladium and carrot taproots (100%) developed a watery soft rot at the site of inoculation that progressed outward in all directions. Infections produced white mycelia and multiple sclerotia. *Sclerotinia sclerotiorum* was isolated from the leading edge of the watery soft rot in carrot and caladium, but could not be isolated from inoculated canna rhizomes or elephant ear corms.

Discussion

This study demonstrates for the first time that elephant ear and canna have significant resistance to white mold caused by *S. sclerotiorum*. Although the pathogen was able to colonize both plants to some degree, neither plant developed wilt or dieback in field or controlled environments. This is also the first report of susceptibility of caladium cultivars to white mold. The petioles, leaves, and tubers of tested cultivars were readily colonized by *S. sclerotiorum*, resulting in soft rot and production of sclerotia. Wilt and death of the canopy and the progression of the infection into tubers makes these cultivars of caladium unsuitable for use in landscape beds infested with *S. sclerotiorum*.

A significant difference in disease severity was observed between the two caladium cultivars in controlled environments, suggesting that there may be varying levels of resistance to *S. sclerotiorum* within cultivars of caladium. The pedigrees of many cultivars of *Caladium ×hortulanum* are unknown, but most are believed to originate from *C. bicolor*, *C. picturatum*, *C. marmoratum*, or *C. schomburgkii* (Deng et al., 2007). Future studies could be conducted to identify resistance in other caladium cultivars or species.

Canna is the sole genus in the Cannaceae (Prince, 2011). Modern day cultivars of *C. ×generalis* are complex interspecific hybrids involving: *C. indica*, *C. glauca*, *C. iridiflora*, and *C. warscicizzi* (Khoshoo and Mukherjee, 1970). Although this study showed high levels of resistance in *Canna* to *S. sclerotiorum*, the study was limited to two varieties. Future studies should examine a broader range of varieties to determine if resistance is consistent across multiple varieties. In addition, orchid cannas (*C. ×orchiodes)*, a complex hybrid involving the four previous species in *C. xgeneralis* plus *C. flaccida* (Khoshoo and Mukherjee, 1970) should be tested for susceptibility to *S. sclerotiorum*. It may be possible to determine the source of resistance by testing each parent species for resistance.

The results of this study show that susceptibility is not consistent in all members of the Araceae. Caladium was highly susceptible to infection, suffering soft rot of leaves, petioles, and corms, whereas elephant ear was highly resistant to infection by *S. sclerotiorum*. The pathogen infected only wounded or senescent tissue and did not result in significant disease symptoms under any conditions.

The Araceae includes a wide variety of ornamental plants for indoor and garden use, in addition to several important food crops. Within the Araceae, plants in two other genera are known to be susceptible to *S. sclerotiorum*: *Philodendron* spp. and *Epipremnum aureum* (Boland and Hall, 1994). Cabrera et al. (2008) proposed eight subfamilies of Araceae and Cusimano et al. (2011) proposed 44 clades of Araceae. *Philodendron, Caladium,* and *Colocasia* are in subfamily Aroideae. *Epipremnum* is in subfamily Monsteroideae. The four Aroid genera found to be susceptible to white mold are in different clades. This indicates that susceptible plants can be found in multiple subfamilies and that both resistant and susceptible plants occur in subfamily Aroideae. Future studies should examine plants from multiple genera from the same clade to determine if resistance is specific to clade or genus.

This study demonstrates that elephant ear and canna are good candidates for planting in landscape beds infested with *S. sclerotiorum*. Canna varieties come in a wide range of heights, foliage and flower colors, allowing them to be utilized in diverse landscape roles. Due to their size, elephant ear plants could be utilized where space allows. Because the pathogen was able to colonize tissue of both elephant ear and canna, use of these plants in infested landscape beds should include removal of plants from the garden bed at the end of the growing season to minimize overwintering of the pathogen in infected plant debris.

Table 5: Combined area under disease progress curve (AUDPC) for incidence and severity of white mold in field-inoculated ornamental tropical plants in 2012 and 2013.

Plant	Cultivar	Incidence AUDPC	Severity AUDPC
Zinnia elegans ×angustifolia	Profusion White	170 a[z]	3234.5 a
Caladium ×hortulanum	White Queen	78.4 b	129 b
Canna ×generalis	Tropical White	81.6 b	4.75 c
Colocasia esculenta	Ruffles	0 c	0 c

[z]Values within a column with the same letter are not significantly different at $\alpha = 0.001$.

Table 6: Percent foliage wilted or killed at 24 days after inoculation by *Sclerotinia sclerotiorum* in a controlled environment.

Scientific Name	Cultivar	Treatment	Percent Leaf Death
Caladium ×hortulanum	White Queen	*S. sclerotiorum*[Z]	65.4 a[y]
Caladium ×hortulanum	Fannie Munson	*S. sclerotiorum*	38.9 b
Colocasia esculenta	Ruffles	*S. sclerotiorum*	21.1 bc
Colocasia esculenta	Ruffles	mock-inoculated control	20.7 bc
Canna ×generalis	Tropical Yellow	*S. sclerotiorum*	1.6 c
Canna ×generalis	Tropical White	*S. sclerotiorum*	0.9 c
Canna ×generalis	Tropical White	mock-inoculated control	0.0 c
Caladium ×hortulanum	White Queen	mock-inoculated control	0.0 c
Canna ×generalis	Tropical Yellow	mock-inoculated control	0.0 c
Caladium ×hortulanum	Fannie Munson	mock-inoculated control	0.0 c

[z]Data from inoculation with three different *S. sclerotiorum* isolates was combined after analysis revealed no significant difference in disease severity between isolates and no interaction between plant entry and isolate.

[y]Values with the same letter are not significantly different at $\alpha = 0.05$.

Table 7: Percentage of below ground storage organ rotted at 10 DAI after wound inoculation with *S. sclerotiorum* isolate ORN010.

Scientific name	Cultivar	Storage Organ	Percent plants infected	Percent storage organ tissue rotted
Canna ×generalis	Tropical White	Rhizome	0 b[z]	0 b
Colocasia esculenta	Ruffles	Corm	0 b	0 b
Caladium ×hortulanum	White Queen	Tuber	25 b	11 b
Daucus carota subsp. *sativus*	unknown	Taproot	100 a	48 a

[z]Values with a column with the same letter are not significantly different at $\alpha = 0.001$.

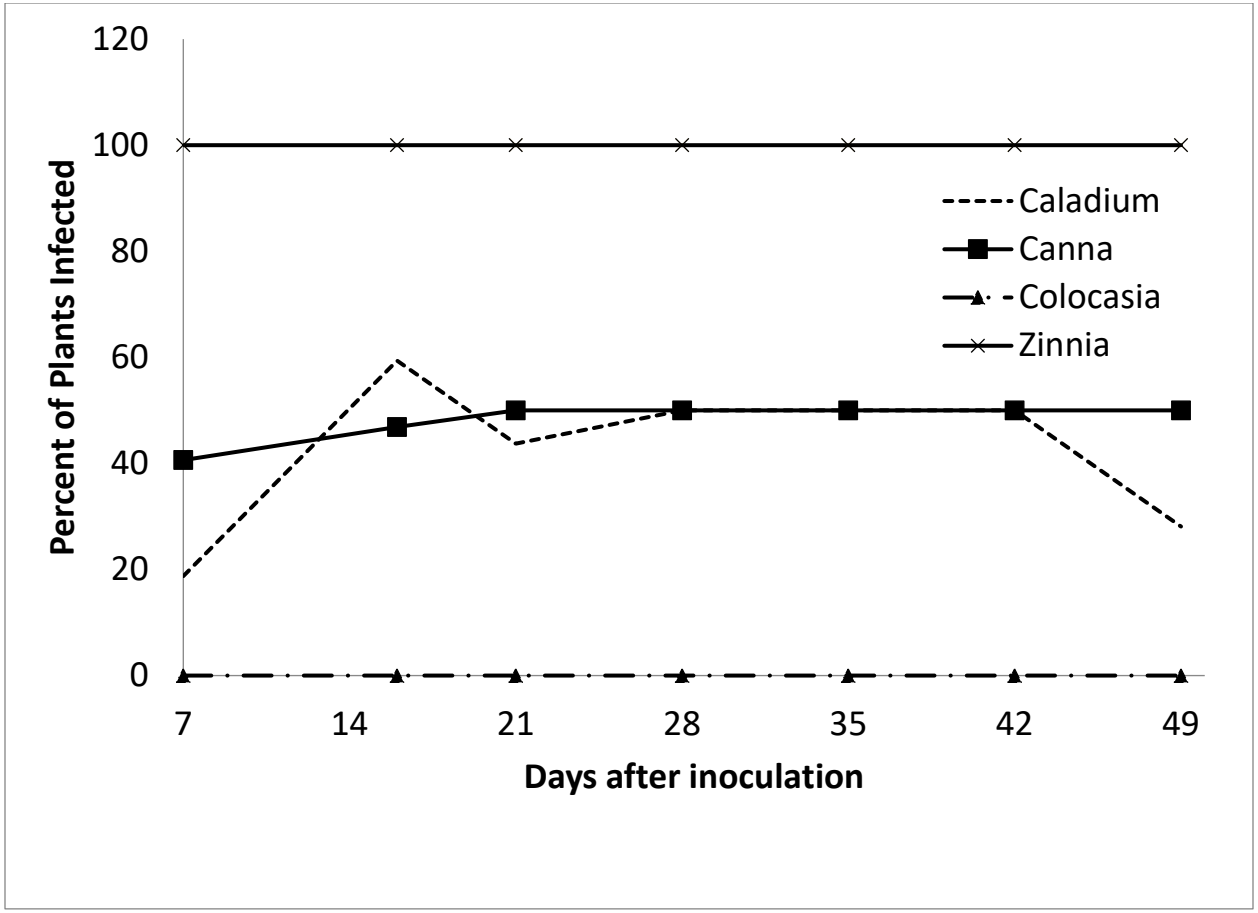

Figure 2: Disease incidence, measured as percent of plants infected by *Sclerotinia sclerotiorum* over time based on signs and symptoms; combined data from 2012 and 2013 field trials.

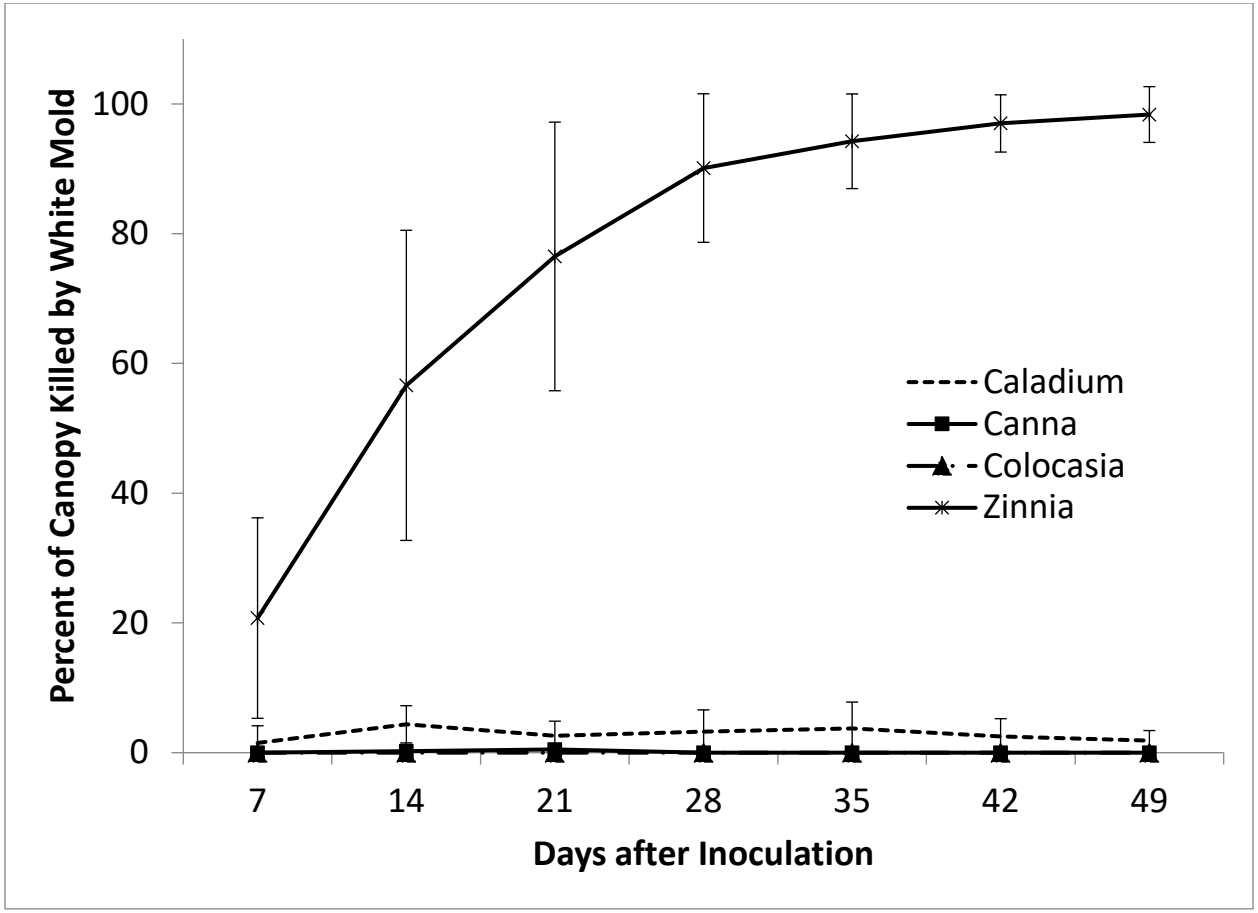

Figure 3: Disease severity, measured as percentage of canopy killed by *Sclerotinia sclerotiorum* over time; error bars represent standard deviation; combined data from 2012 and 2013 field trials.

Chapter 4

Evaluating ornamental grasses for resistance to white mold: exploring the host range of *Sclerotinia sclerotiorum* in graminoids

Synopsis. *Sclerotinia sclerotiorum* is a necrotrophic fungal plant pathogen known to infect over 400 species of plants from 75 families. Many popular annual flowering plants are susceptible, resulting in mid-season plant death. The objective of this study was to determine if the ornamental graminoids *Pennisetum glaucum, Setaria italica, Juncus inflexus, Carex flagellifera, Isolepsis cernua,* and *Acorus gramineus* are resistant to *S. sclerotiorum*. To determine resistance in field environments, plants were inoculated with *S. sclerotiorum* at two locations and were evaluated for disease incidence and severity. To determine the effect of temperature on resistance, plants were maintained in a growth chamber at 13, 16, 19, or 22^{o}C after wound inoculation of stems. *Juncus inflexus, P. glaucum,* and *A. gramineus* were also inoculated with or without wounding in a growth chamber at 13^{0}C. Lesion length was measured 28 days after inoculation. To determine the pathogen's ability to penetrate leaf tissue and induce production of oxalate oxidase, detached leaves were inoculated and stained. *Acorus gramineus* was susceptible to *S. sclerotiorum* in field and growth chamber environments, regardless of wounding and temperature. *Juncus inflexus, C. flagellifera,* and *I. cernua* were resistant with minor to no symptom development in field and controlled environment studies. The reaction of *P. glaucum* and *S. italica* to inoculation varied under different conditions. Disease severity increased as temperature decreased and with wounding. Although *P. glaucum* was resistant in field conditions, this species was susceptible when wounded and maintained at 13^{0}C. Mycelial penetration of leaf tissue occurred 24 hours after inoculation (hai) for the susceptible entry *A. gramineus*, 48 hai for the intermediate resistant entry *P. glaucum,* and was not observed at 24, 48, or 96 hai for the resistant plant entry *J. inflexus*. Oxalate oxidase, a plant defense compound found in other graminoid species, was not observed in

association with infection of *A. gramineus, P. glaucum, J. inflexus, C. flagellifera,* or *I. cernua.* Results from these studies document that the ornamental graminoids differ in susceptibility to *S. sclerotiorum*. This study demonstrates different levels of resistance to *S. sclerotiorum* in ornamental graminiods. *Juncus inflexus, C. flagellifera,* and *I. cernua* are resistant to *S. sclerotiorum* and useful in infested landscape beds.

Introduction

Sclerotinia sclerotiorum 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 ornamentals (Daughtrey et al., 1995; Gleason et al., 2009; Hartman et al., 2015; Harveson et al., 2016; Jones et al., 2014; Koike, 2007; Rimmer et al., 2007; Schwartz et al., 2005). The disease is commonly known as Sclerotinia stem rot or white mold. Although symptoms vary between hosts, infection commonly progresses into the plant stem resulting in wilt and death of all parts distal to the infection. Sclerotia produced by *S. sclerotiorum* can survive up to 8 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 and production of long term survival structures combined with high reproductive rates and aerial dissemination of spores make *S. sclerotiorum* a challenging pathogen to manage.

In ornamental landscape beds, white mold causes stem rot, wilt, and death of many common annual bedding plants including zinnia, petunia, snapdragon, verbena, and salvia (Boland and Hall 1994; Farr and Rossman 2017). 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; an unacceptable condition for high value, high visibility landscape beds.

Although ornamental plants are not bred for resistance to white mold, one management option available to landscape managers is to choose plant species that are outside the host range of *S. sclerotiorum*. The complete host range of *S. sclerotiorum*, however, is not well defined. Over 400 species of plants from 75 families are known to

be susceptible to white mold (Boland and Hall 1994; Farr and Rossman 2017). Daughtrey et al. (1995) reported 10 genera of common flowering potted plants. Gleason et al. (2009) listed over 30 genera of herbaceous perennials that are susceptible to *S. sclerotiorum*. Boland and Hall (1994) list over 50 susceptible genera in the Asteraceae alone. These lists, however, are outdated as reports of new hosts have been published (Chang et al., 1997; Garibaldi et al., 2001, 2008a, 2008b, 2008c, Grabowski and Malvick 2015, 2017, Gulya et al., 2006; Strauss and Dillard 2009).

The host range of *S. sclerotiorum* includes dicotyledons and monocotyledons, although most known hosts are dicots. Of the five families of monocotyledonous plants listed as hosts to *S. sclerotiorum* (Boland and Hall 1994), the only graminiod family is the Poaceae. The two most recently published host lists do not agree on which members of the Poaceae are hosts. Boland and Hall (1994) reported plants from nine genera of Poaceae as hosts of *S. sclerotiorum*, whereas Farr and Rossman (2017) list plants in eight genera as hosts. Four genera (*Zea, Sorghum, Setaria, Triticum*) are found on both 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 rotation with susceptible crops (Bockus et al. 2010; Warren and Frederiksen 1986; White and Munkvold 2016). Plants 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; Miclauş et al. 1988; Richardson 1990). Plants within many genera of the Poaceae appear on published disease indices from various locations and do not include a description of symptoms or severity of the infection (Conners 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 gramioids remains unclear.

Use of ornamental grasses in landscape beds infested with *S. sclerotiorum* has been proposed as a means to avoid damage from white mold. However, although grasses are often assumed to be non-hosts of *S. sclerotiorum,* little is known about the susceptibility and resistance of ornamental grasses to white mold. Annual ornamental grasses in the Poaceae, Cyperaceae, Juncaceae and Acoraceae are readily available to landscape managers. None have been tested for resistance or susceptibility to *S. sclerotiorum*. The objective of this study was to determine how plants in six genera from these four families of ornamental grasses react macroscopically and microscopically to inoculation by *S. sclerotiorum* under different environmental conditions. Evaluation of resistance and susceptibility of ornamental grasses not only provides information on which ornamental grasses have potential to be successfully grown in infested landscape beds, but also contributes to the understanding of *S. sclerotiorum*'s host range within monocotyledons.

Materials and Methods

***Isolate preparation*.** Three isolates of *S. sclerotiorum* from diverse hosts were arbitrarily chosen for this study. The isolates were ORN010, isolated from a petunia plant in Chaska, MN; FC002, isolated from a soybean plant in Renville County, MN; and VEG001, isolated from green bean in Spring Valley, WI. All isolates were grown and stored as described in Grabowski and Malvick (2015).

Plant materials. Annual ornamental grasses widely available to landscape managers and representing a diversity of families and genera were selected for use in the studies. These included species in six genera of graminoids from four families (Table 8). Seed of *P. glaucum* and *S. italica* (Johnny's Selected Seeds, Fairfield, Maine) were planted in 10.2 cm pots containing a composted bark potting mix (Sungro Horticulture, Agawam, MA) and maintained in a greenhouse with 12 h light at 25 ^{0}C and 12 h darkness at 20 ^{0}C. Seedlings were fertilized by watering pots to saturation one week after emergence with 20-20-20 soluble fertilizer diluted to 1.2 g/L (Everris NA, Inc. Dublin, OH). Osmocote 14-14-14 (Everris NA, Inc.) was added to all pots 2 weeks after emergence at 0.0048 g/cm^3. Plants were grown for approximately 10 weeks until 8-10 fully expanded leaves were present. *Juncus inflexus, I. cernua, A. gramineus,* and *C. flagellifera* were purchased as transplants from a garden center (Linder's Greenhouses Inc., Roseville, MN) in 10.2 cm pots. *Zinnia elegans* × *angusifolia* 'Profusion White' transplants (Linder's Greenhouses Inc.) were included in field trials as a susceptible control. All plants were maintained in a greenhouse with 12 h light at 25 ^{0}C and 12 h darkness at 20 ^{0}C prior to planting in field trials.

Field studies of disease susceptibility. The study was set up in a randomized complete block design with six blocks, and repeated at two locations containing Waukegan silt loam on the Minnesota Agricultural Experiment Station, St. Paul, MN in 2011. Plants were transplanted into the study at one location on 8 June and at the other location on 14 June. Experimental units were 107 cm x 107 cm beds with nine plants arranged in three rows of three plants and 35.5 cm between plants. A single row of

‘Profusion Cherry’ zinnias was planted as a border around each block to help maintain even moisture within the beds and reduce edge effects. Plants were watered daily for 20 minutes with an oscillating sprinkler to promote establishment prior to inoculation. Osmocote 14-14-14 (340 kg/ha) was applied to all plots 2 weeks after planting.

Plots were inoculated with a mixture of equal quantities of sorghum seed colonized by the three isolates of *S. sclerotiorum,* prepared as described by Grabowski and Malvick (2015). The inoculum mixture was spread evenly over the plant canopy at 86.5 L/ha two times, once on August 4, 2011 when the susceptible control plants ‘Profusion White’ zinnia had grown together to achieve canopy closure, and again one week later. Irrigation was applied for 5 min immediately after inoculation and was subsequently applied three times daily for 10 min for the remainder of the experiment. Following the inoculations, the daily maximum temperature ranged from 22.7 ^{0}C to 29 ^{0}C and the minimum temperature ranged from 15.5 ^{0}C to 23 ^{0}C during the study.

Disease severity and incidence were recorded every 14 days after inoculation (DAI) until frost. Disease incidence was recorded as the number of plants per bed that were visibly infected with *S. sclerotiorum* based on signs and symptoms. The leading edge of the infection from any plant with questionable symptoms was surface sterilized and plated on half strength PDA (1/2x PDA, Becton, Dickinson and Company, Franklin Lakes, NJ) to confirm infection by *S. sclerotiorum*. Disease severity was recorded as percent canopy missing due to death of plants within the bed. The area under disease progress curve (AUDPC) (Madden et al., 2007) was calculated 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 delayed planting and an early frost in field 2.

Studies of disease susceptibility at different temperatures under controlled conditions. Plant entries included the six grown as described above. Plants were evaluated for resistance under five different temperature regimes. The experiments were set up as a randomized complete block design with three blocks. Only *S. sclerotiorum* isolate ORN010 was used for growth chamber studies because previous studies demonstrated no difference in virulence or host x isolate interactions for the three isolates of *S. sclerotiorum* utilized in the field study (Grabowski and Malvick 2015, 2017). Sclerotia were placed individually on 1/2x PDA 5 days prior to the experiment and incubated at 22 ^{0}C to promote mycelial growth. An 8 mm square of 1/2X PDA containing the leading edge of the colony was secured to a lower stem of each plant with a sterile toothpick inserted approximately 0.6 cm into the stem. Mock inoculated plants were treated in a similar fashion but received only sterile 1/2X PDA. All plants were thoroughly watered, 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 13, 16, 19, or 22 ^{0}C with a 12 h photoperiod, and 90% humidity. Bags were removed 3 DAI. At 28 DAI, percent of foliage wilted or killed was recorded, plants were cut at soil level and vertically bisected, and internal and external lesion length was measured. The experiment was performed twice at each temperature.

Studies of disease susceptibility in response to wound inoculation. *Pennisetum glaucum*, *J. inflexus*, and *A. gramineus* 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, and was repeated twice at different times. Treatments included basal stem inoculation with wound, basal stem inoculation without

wound, leaf inoculation with wound, leaf inoculation without wound, 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 both wounded and unwounded plants. Leaves were wounded by removing the distal half of a fully expanded leaf with a sterile razor blade, and an agar plug, prepared as described above, was placed on the cut edge. In unwounded leaves, the agar plug was placed on the adaxial surface at the midpoint of the leaf. In both wounded and unwounded leaves, the agar plug was attached with Parafilm®. Mock inoculated controls were wounded at the leaf and stem as described above and sterile 1/2X 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 maintained in a growth chamber set to 13 ^{0}C with 12 h light, 12 h darkness, and 90% humidity. Lesion length and number of leaves killed due to infection was determined 28 DAI. The leading edge of the infection from plants with questionable symptoms was plated on ½x PDA and incubated 14 days at 22 ^{0}C to determine if the pathogen was present.

Microscopic examination of leaf response to inoculation. *Pennisetum glaucum*, *J. inflexus*, and *A. gramineus* plants were grown in the greenhouse as described above. Fully expanded leaves were harvested and a 6 cm section was cut from the middle of a leaf with a sterile razor blade and placed on wet, sterile filter paper (Whatman International, Maidenstone, England) in a sterile 100 mm x 15 mm petri dish. An agar

plug of *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 only sterile ½x PDA. Petri dishes were sealed with Parafilm® and incubated at 22 ^{0}C.

To determine if *S. sclerotiorum* created infection cushions on non-plant surfaces, an agar plug of *S. sclerotiorum* isolate ORN010, prepared as described above, was placed at the center of a glass microscope slide, a glass slide wrapped in labeling tape (Fisherbrand, Houston, TX), and a glass slide containing a 2.5 x 3 cm piece of colored construction paper (Wexford, Deerfield, IL) saturated with Millipure filtered water and placed in the center of the slide. The mock inoculated slides were sealed in petri dishes and incubated as described above.

Leaves were examined at 24 and 48 hai for all plant entries. *Juncus inflexus* leaves were also examined at 96 hai because no evidence of penetration had been observed at earlier time intervals. 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. In addition, 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. Plants entries (Table 8) were grown as described above. *Triticum aestivum* 'Morocco' and *Hordeum vulgare* 'Bowman' were also included in the experiment as positive controls. Seed of wheat and barley were planted in 10.2 cm pots and grown as described for *P. glaucum*

and *S. italica* above. Fully expanded leaves were harvested from trial plants. Eight, 6 cm long sections of tissue were cut from the middle of the leaves with a sterile razor blade and placed on filter paper in a petri dish as described above. An agar plug of *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 only sterile 1/2xPDA. Petri dishes were sealed with parafilm® (Bemis Company, Inc., Oshkosh, WI) and incubated at 22 ^{0}C for 1, 4, 6, or 24 h. One inoculated and one mock inoculated leaf segment was removed per time interval. Agar plugs were removed and leaf tissue was transferred into 1.2 ml glass vials containing 1 ml of oxalate oxidase detection buffer (Orshinsky et al. 2012; Dumas et al. 1995) for 1 h at 22 ^{0}C. Leaves were removed and examined with a Nikon eclipse NiU light microscope for dark blue staining indicative of oxalate oxidase activity. This experiment was conducted twice.

To determine if the cuticle was interfering with the 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, the agar plug was removed and one leaf per plant entry was placed into the oxalate oxidase detection buffer for 1 h and examined as described above. One leaf segment per plant entry was also 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. Normal quantile plots, histograms, and residuals plots were used to determine that data from field experiments and wound inoculation trials were 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 non-parametric methods were used to analyze the data. Friedman's test was used to calculate a chi-squared 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. It was determined that variances were homogeneous between repeated trials 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.

Results

Studies under field conditions. Disease incidence and severity 14 DAI of the susceptible control 'Profusion White' zinnia ranged from 56 to 100% and 9 to 80%, respectively indicating favorable conditions for disease development. The annual ornamental grasses, *J. inflexus, I. cernua, C. flagellifera,* and *P. glaucum* remained symptom free throughout the experiment. *Acorus gramineus* was the only grass to develop white mold symptoms under field conditions. An elongate tan lesion developed on the rhizomes, infection spread into leaf clusters, leaves turned tan and died. *Setaria italica* plants died shortly after transplant and were not analyzed further. The AUDPC for disease incidence and severity for all grasses was significantly lower than the susceptible control (Table 9).

Studies of disease susceptibility at variable temperatures. The effect of temperature on disease severity varied by plant entry. Differences in internal and external stem lesion length among plant entries were significant and most easily discernable at 13 ^{0}C (Table 10). Percent foliage killed was confounded by natural leaf senescence in some species and was not significantly different between mock inoculated and inoculated plants for any plant entry (data not shown).

Isolepsis cernua, J. inflexus, and *C. flagellifera* exhibited no symptoms or minor symptoms of disease at all temperatures (Table 10). There was no significant difference between inoculated and mock inoculated plants for external or internal stem lesion length at any temperature (Table 10). Lesion length was lower for these species than for inoculated *A. gramineus*, *P. glaucum,* and *S. italica* at 13 and 16 ^{0}C (Table 10). Internal and external lesion length of *C. flagellifera, I. cernua,* and *J. inflexus* was unaffected by temperature (Fig. 4 and 5). Small, brown lesions (<1.0 cm) were observed on 8 and 21% of inoculated *C. flagellifera* and *J. inflexus* plants, respectively and *S. sclerotiorum* was isolated from 8% of these plants. A small sclerotium was found inside 8% of *C. flagellifera* and *J. inflexus* stems (Fig. 6). No signs or symptoms were observed in inoculated *I. cernua* plants and the pathogen was not isolated from these plants.

Acorus gramineus, P. glaucum, and *S. italica* were susceptible to *S. sclerotiorum* at all temperatures, but the effect of temperature on disease severity varied by plant entry (Table 10). Lesion length for *P. glaucum* and *S. italica* increased exponentially as temperature decreased, and was significantly greater at 13 ^{0}C than at all other temperatures (Fig. 4 and 5). 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 (Fig. 7). Lesion length for inoculated *A. gramineus* did not differ between 13, 16, and 19 ^{0}C, but was significantly lower and did not differ from mock inoculated controls at 22 ^{0}C (Figure 4 and 5, Table 10). An elongate tan lesion developed on *A. gramineus* rhizomes, infection spread into leaf clusters, and leaves turned tan and died (Fig. 8). Mycelia and sclerotia were observed in infected leaf clusters.

Studies of disease susceptibility in response to wound inoculation. The effect of wounding on disease severity varied by plant entry. Infection of *A. gramineus* plants occurred regardless of wounding, and lesion length did not differ between wounded and unwounded plants (Table 11). Lesion length on inoculated *P. glaucum* leaves was similar in wounded and unwounded plants, and leaf infections never progressed into stem tissue 28 dai (Table 11). Dark brown lesions were observed on the surface of stem inoculated *P. glaucum* plants in wounded and non-wounded treatments, but infection progressed into stem pith tissue only in wounded stems (Table 11, Fig. 7). *Juncus inflexus* plants only developed a measurable necrotic lesion when wounded prior to inoculation (Table 11, Fig. 6). All of the *J. inflexus* stems inoculated through an apical wound became infected, and 33% of stems inoculated through a basal wound became infected.

Microscopic examination of the infection site. *Sclerotinia sclerotiorum* attempted to infect *J. inflexus*, *P. glaucum*, and *A. gramineus* as was shown by the presence of multiple infection cushions on the leaf surface of all inoculated plant entries at 24 and 48 hai (Fig. 9). Infection cushions did not develop on inoculated glass slides, glass slides with lab tape, or glass slides with construction paper (Fig. 9).

The ability of *S. sclerotiorum* to infect and the rate of infection varied among plant entries. Infection and colonization of epidermal cells, mesophyll cells, and elaioplasts of *A. gramineus* was observed at 24 hai (Fig. 10). *Acorus gramineus* 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 (Fig. 10). No penetration of *J. inflexus* leaf tissue was observed at 24, 48, or 96 hai, although multiple infection cushions were present at all three time points. By 96 hai infection cushions were degraded, flattened, and lacked structure (Fig. 10). By 96 hai, the cuticle, mesophyll cells, and sclerenchyma below the infection cushion were discolored brown but remained intact and turgid (Fig. 10).

Examination of oxalate oxidase activity in inoculated leaf tissue. Oxalate oxidase production was detected in association with infection by *S. sclerotiorum* in wheat and barley at 24 hai, but was not detected in *J. inflexus*, *I. cernua*, *C. flagellifera*, *P. glaucum*, or *A. gramineus* at any time point. Although necrotic lesions developed at the site of inoculation for *A. gramineus*, *S. italica*, wheat, and barley, lesion development only corresponded to detection of oxalate oxidase in wheat and barley. Dark staining was observed macroscopically at the edge of lesions in barley and wheat. No staining was observed macroscopically in *S. italica*, but minor staining was observed microscopically in cell walls at the edge of the lesion.

Cuticle removal did not affect either lesion development or oxalate oxidase staining. No staining was observed in plant cells at the inoculation point of *A. gramineus, J. inflexus, I. cernua, P. glaucum, or C. flagellifera* regardless of cuticle removal. Dark

staining was observed at the lesion edge of all wheat leaf samples regardless of cuticle removal.

Discussion

This study is the first report that *C. flagellifera, J. inflexus,* and *I. cernua* are resistant to infection by *S. sclerotiorum*. No disease was observed under field conditions on these three species. Infection of *C. flagellifera* and *J. inflexus* occurred rarely with wound inoculation in a controlled environment but these infections were restricted to the inoculation sites. Despite the natural dense humid canopy of *I. cernua*, symptoms of white mold were not observed in field or controlled environment conditions, nor was the pathogen recovered from inoculated plant tissue in any experiment. *Carex flagellifera, I. cernua,* and *J. inflexus* are good candidates for use in landscape beds infested with *S. sclerotiorum*. Further study of the defense mechanisms utilized by these three graminoids could prove useful for breeding resistance to *S. sclerotiorum*. The resistant plant entries identified in this study are members of the Cyperaceae and Juncaceae. Further research is necessary to determine if *S. sclerotiorum* can infect other species within these two families.

This study is also the first report of susceptibility of *A. gramineus* and *S. italica* to white mold. A different species of *Setaria*, *S. viridis,* is listed as a host to *S. sclerotiorum* (Boland and Hall 1994; Farr and Rossman 2017). *Setaria viridis* is a common grassy weed found in agricultural fields throughout North America. Burke et al. (1957) observed water soaked stem lesions on *S. viridis* in bean fields in Colorado, and symptomatic stems

developed sclerotia of *S. sclerotiorum* after incubation. In contrast, Fick and Gulya (1980) reported that in heavily infected sunflower fields, *S. viridis* showed no symptoms of infection even though five other weed species had clear white mold symptoms. The symptoms of *S. italica* observed in this study were similar to those of *S. viridis* (Burke et al. 1957). This study demonstrates an increase in disease severity with decreasing temperature for *S. italica* plants. The effects of temperature on susceptibility in *S. viridis* has not been reported previously, but the contradictory reports of susceptibility to white mold could be due to differences in temperature between the two studies.

Pennisetum glaucum appears on one of two host lists for *S. sclerotiorum* (Boland and Hall 1994; Farr and Rossman 2017). The cited reference only indicates that *P. glaucum* seed could serve as a growth medium for the pathogen in a laboratory experiment (Singh and Singh 1983). This present study confirms that *S. sclerotiorum* is capable of infecting stems and leaves of *P. glaucum* plants, and that symptoms are similar to those reported for *S. viridis* (Burke et al. 1957). Susceptible plant entries in this study are members of the Poaceae and Acoraceae. This is the first report of a member of the Acoraceae as a susceptible host to *S. sclerotiorum.* Further research is needed to determine the full extent of the host range of *S. sclerotiorum* within these graminoid families.

Temperature had differing effects on white mold in plant species included in this study. Disease severity for the three highly resistant plants, *C. flagellifera*, *J. inflexus*, and *I. cernua*, was consistent across a range of temperatures. However, disease severity in the three susceptible plant entries varied with temperature. While disease severity of *P. glaucum* and *S. italica* increased with decreasing temperature, disease severity of *A.*

gramineus was consistent from 13 to 19 ^{0}C and decreased significantly at 22 ^{0}C. Optimal growth of *S. sclerotiorum* in culture and maximum lesion length in bean occurs at 20-25 ^{0}C (Abawi and Grogan 1975). Increases in disease severity due to temperature did not correspond to conditions for optimal growth of the pathogen, nor was it consistent among plant species. This indicates that temperature likely affects host defense mechanisms. Further trials should be conducted to determine if the relationship between disease severity and temperature is consistent across multiple isolates of *S. sclerotiorum*. This temperature dependent response to infection is important to take into account when screening plants for resistance. New species or varieties should be tested over the range of temperatures likely to occur in production to verify the stability of defense mechanisms at variable temperatures.

Observations from microscopic studies supported the results from the field and growth chamber studies. *Acorus gramineus* was susceptible to infection in field and growth chamber experiments. Mycelial penetration and colonization of leaf tissue occurred 24 hours after inoculation (hai) for this species. This parallels the observations made of susceptible bean varieties where penetration and colonization of host tissue was observed 24 hai (Abawi et al. 1975; Lumsden and Dow 1973). These results indicate that *A. gramineus* does not possess host defense mechanisms that prevent or slow infection by *S. sclerotiorum*. Although *A. gramineus* became infected and expressed symptoms of white mold, disease severity was significantly lower than the susceptible control in all studies and never progressed to plant death. It is possible that defense mechanisms in *A. gramineus* reduce the growth and spread of *S. sclerotiorum* in planta.

Penetration and colonization of *P. glaucum* was not observed until 48 hai despite the presence of infection cushions at 24 hai. In wound trials, *S. sclerotiorum* was unable to progress into pith tissue without wounding. These results indicate that *P. glaucum* possesses defense mechanisms that reduce *S. sclerotiorum*'s ability to infect. In addition, *P. glaucum* likely expresses a defense mechanism that reduces the growth and spread of *S. sclerotiorum* in planta in a temperature dependent fashion, as is evidenced by the significant increase in lesion length observed at 13 ^{0}C.

Penetration and colonization of plant tissue was not observed at 24, 48, or 96 hai for the resistant plant entry *J. inflexus*. Histological studies have shown that *S. sclerotiorum* forms domed infection cushions of mounded mycelia with multiple penetration hyphae positioned perpendicular to the host surface to initiate an infection (Lumsden and Dow 1973; Lumsden and Wergin 1980). In detached leaf assays in this study, *S. sclerotiorum* formed infection cushions on *J. inflexus* but not on any of the mock leaf surfaces. This indicates that *S. sclerotiorum* recognized *J. inflexus* as a potential host but failed to initiate infection.

Infection of *J. inflexus* was observed in wound inoculated plants, but progression of disease was limited. These results indicate that *J. inflexus* possesses defense mechanisms that significantly reduce the ability of *S. sclerotiorum* to colonize host tissue. Discoloration of the cuticle, epidermal, and mesophyll cells was observed at the infection site in *J. inflexus*, indicating a change in chemistry that likely includes production of plant defense compounds. Upregulation of numerous plant defense mechanisms, including phenols, phytoalexins, pathogenesis related proteins, cell wall degrading enzymes, and proteins associated with the oxidative burst, has been recorded in disease 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). Further research is needed to determine the nature of defense mechanisms utilized by the resistant plants identified in this study.

Oxalic acid has been identified as an important pathogenicity factor for *S. sclerotiorum* (Cessna et al. 2000; Godoy et al. 1990; Guimaraes and Stotz 2004; Noyes and Hancock 1981; Marciano et al. 1983). Wheat and barley produce oxalate oxidase as a defense response against fungal pathogens (Hurkman and Tanaka 1996; Zhou et al. 1998). Transgenic expression of the oxalate oxidase gene into susceptible crops has resulted in increased resistance to *S. sclerotiorum* (Cober et al. 2003; Donaldson et al. 2001; Hu et al. 2003). Although oxalate oxidase production was detected in wheat and barley leaves inoculated with *S. sclerotiorum*, no oxalate oxidase production was detected in any of the highly resistant graminoids identified in this study. This indicates that different defense responses play a role in protecting *C. flagellifera*, *J. inflexus*, and *I. cernua* against *S. sclerotiorum*.

The resistant ornamental graminoid species identified in this study may be good options for use in landscape beds infested with *S. sclerotiorum*. Further studies of the resistant hosts identified in this study could provide valuable information about their defense mechanisms against white mold. Although traditional breeding would not be an option to integrate these defense mechanism into susceptible plant species, transgenic approaches could be potentially be utilized.

Table 8: Annual ornamental grasses evaluated for susceptibility to *Sclerotinia sclerotiorum*.

Scientific Name	Variety	Common Name	Family
Acorus gramineus	Ogon	Sweet Flag	Acoraceae
Carex flagellifera	Toffee Twist	Sedge	Cyperaceae
Isolepsis cernua (Syn. *Scirpus cernuus)*	Fiber Optic Grass	Low Bulrush	Cyperaceae
Juncus inflexus	Blue Mohawk	Reed	Juncaceae
Pennisetum glaucum (Syn. *Cenchrus americanus)*	Purple Majesty	Millet	Poaceae
Setaria italica	Lime Light Spray	Foxtail millet	Poaceae

Table 9: Area under disease progress curve (AUDPC) for incidence and severity for white mold in field-inoculated annual ornamental grasses.

	Incidence AUDPC		Severity AUDPC	
Scientific name	**Field 1**	**Field 2**	**Field 1**	**Field 2**
Carex flagellifera	0 c[z]	0 c	0 b	0 b
Juncus effusus	0c	0 c	0 b	0 b
Pennisetum glaucum	0 c	0 c	0 b	0 b
Isolepsis cernua	0 c	0 c	0 b	0 b
Acorus gramineus	211 b	146 b	22 b	7 b
Zinnia elegans x angustifolia	3344 a	2048 a	2132 a	942 a

[z]Values with the same letter within a column are not significantly different at $\alpha = 0.001$.

Table 10: External and internal stem lesion length on six ornamental graminoids following inoculation with *Sclerotinia sclerotiorum* and incubation at 13, 16, 19, and 22^{0}C.

Temperature	13 ^{0}C		16 ^{0}C		19 ^{0}C		22 ^{0}C	
	Stem lesion length (cm)							
Plant	External	Internal	External	Internal	External	Internal	External	Internal
Setaria italica	2.37 a[z]	6.02 a	1.55 ab	2.57 a	1.25 ab	1.50 ab	1.13 a	1.02 a
Pennisetum glaucum	2.70 a	4.66 a	1.12 b	1.83 ab	0.85 b	0.93 bc	0.73 ab	0.68 ab
Acorus gramineus	2.73 a	1.80 b	2.03 a	1.58 b	1.77 a	1.82 a	0.52 bc	0.00 c
Juncus inflexus	0.13 b	0.10 b	0.00 c	0.00 c	0.58 bc	0.50 cd	0.43 bc	0.07 c
Carex flagellifera	0.05 b	0.10 b	0.00 c	0.00 c	0.00 c	0.18 cd	0.15 c	0.35 bc
Isolepsis cernua	0.00 b	0.00 b	0.00 c	0.00 c	0.00 c	0.00 d	0.00 c	0.00 c
mock inoc. control*	0.00 b	0.00 b	0.00 c	0.00 c	0.00 c	0.00 d	0.00 c	0.00 c

*All mock inoculated controls had the same value. Although statistical analysis was conducted on individual mock inoculated entries, mock inoculated data is displayed as one entry to conserve space.

[y]Values with the same letter within a column are not significantly different at $\alpha = 0.05$.

Table 11: Lesion length in response to inoculation of wounded or non-wounded leaves and stems of *Juncus inflexus*, *Acorus gramineus*, and *Pennisetum glaucum* with *Sclerotinia sclerotiorum*.

	Lesion length (cm)			
	Juncus inflexus	*Acorus gramineus*	*Pennisetum glaucum*	
			External	Internal
leaf unwounded[z]	0.0 b[y]	12.8 b[x]	0.0 a[y]	0.0 a[y]
leaf wound[z]	5.3 a	11.6 b	0.0 a	0.0 a
stem unwounded	0.0 b	32.1 c	0.9 b	0.0 a
stem wound	0.1 b	25.0 bc	1.9 b	3. b
Uninoculated control	0.0 b	0.0 a	0.0 a***	0.0 a***

[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.

[y]Values within a column with the same letter are not significantly different at $\alpha = 0.001$.

[x]Values within a column with the same letter are not significantly different at $\alpha = 0.01$.

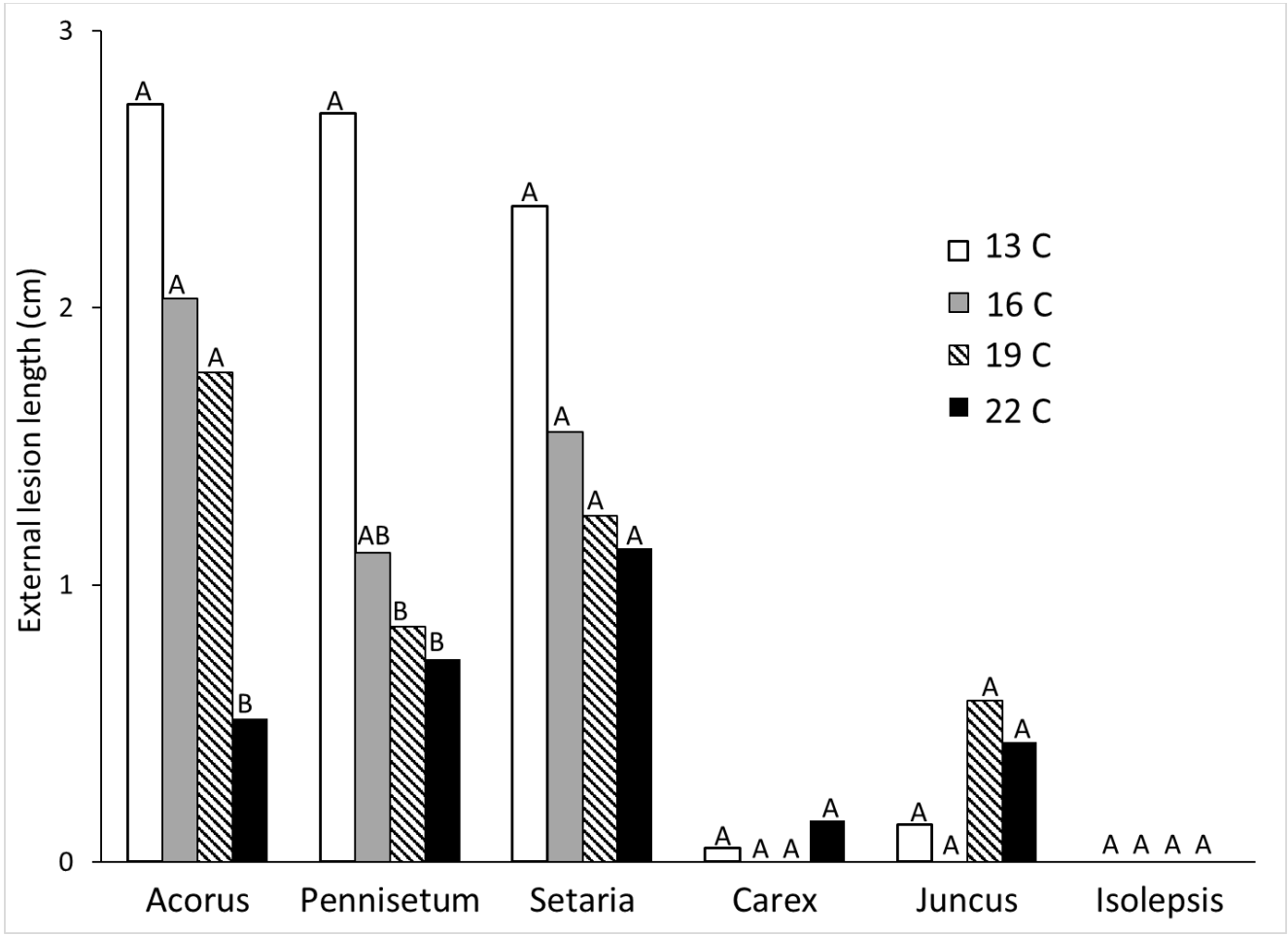

Figure 4: External lesion length on stems of ornamental grasses inoculated with *S. sclerotiorum* at four temperatures. Values with the same letter within a plant entry are not significantly different at $\alpha = 0.01$.

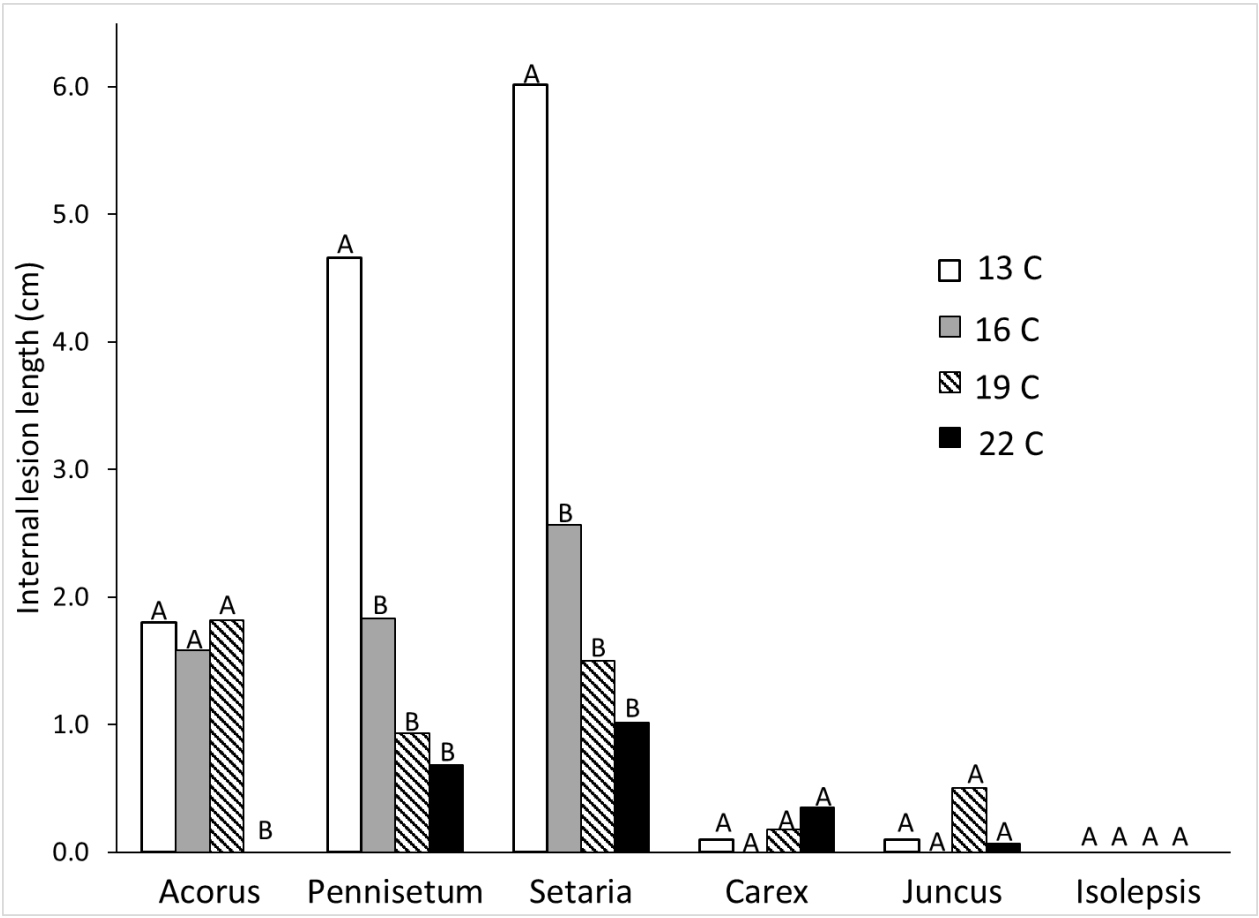

Figure 5: Internal lesion length in stems of ornamental grasses inoculated with *S. sclerotiorum* at four temperatures. Values with the same letter within a plant entry are not significantly different at $\alpha = 0.01$.

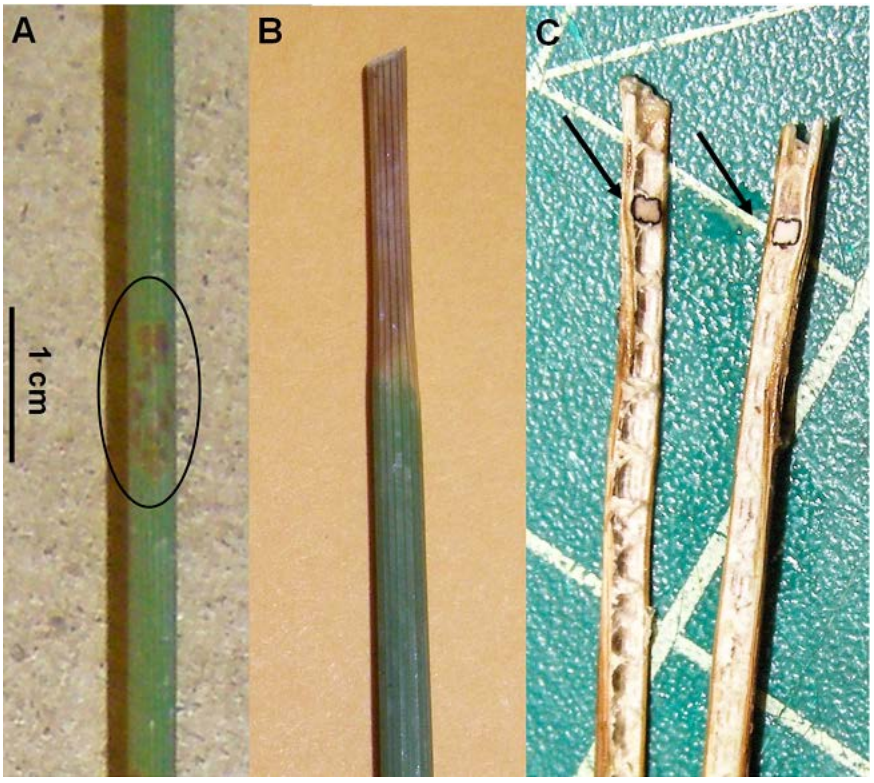

Figure 6: Symptoms that developed on *Juncus inflexus* following inoculation by *Sclerotinia sclerotiorum* at 28 days after inoculation incubated at 13^0C, **A**, apical inoculation without wound; **B**, apical inoculation with wound; **C**, internal sclerotia produced after apical inoculation with wound.

Figure 7: Symptoms of infection of *Pennisetum glaucum* by *Sclerotinia sclerotiorum* at 28 days after inoculation incubated at 13^0C, **A**, tan external stem lesion with sclerotia on wound inoculated stem; **B**, discoloration and degradation of pith, with mycelia and sclerotia on wound inoculated stem; **C**, healthy pith of stem inoculated without wound.

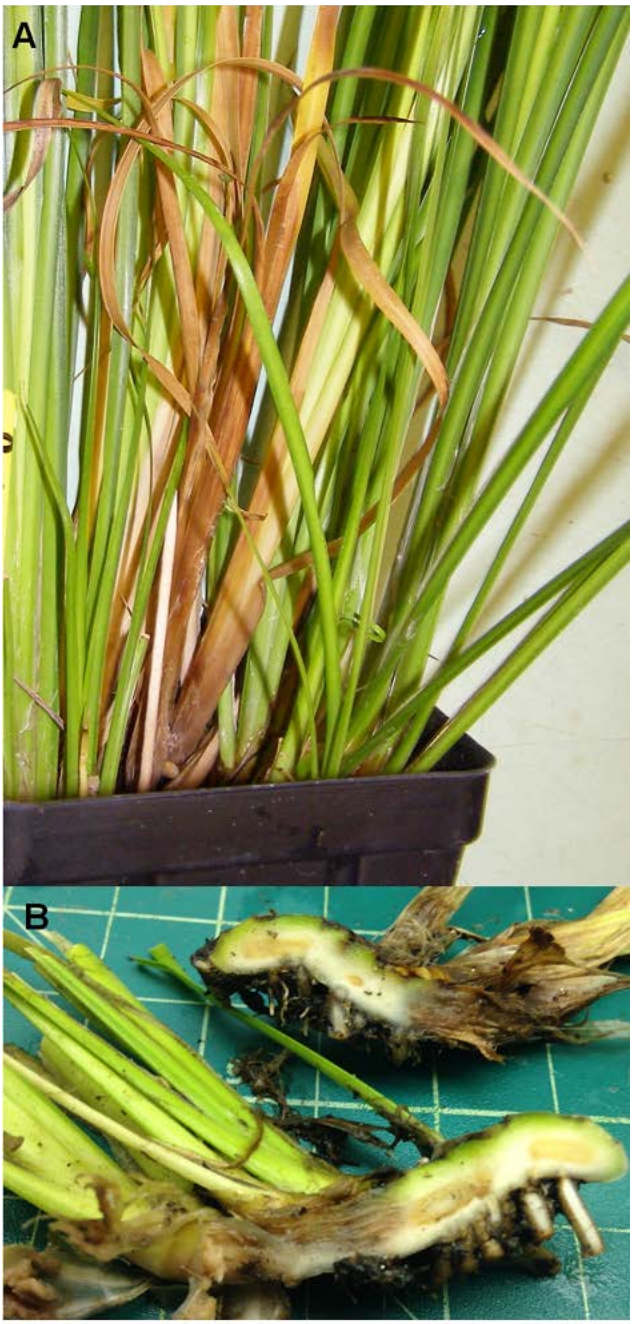

Figure 8: Symptoms of infection of *Acorus gramineus* by *Sclerotinia sclerotiorum* at 28 days after inoculation incubated at 13^0C, **A**, inoculation of rhizome with wound; **B**, internal discoloration of rhizome.

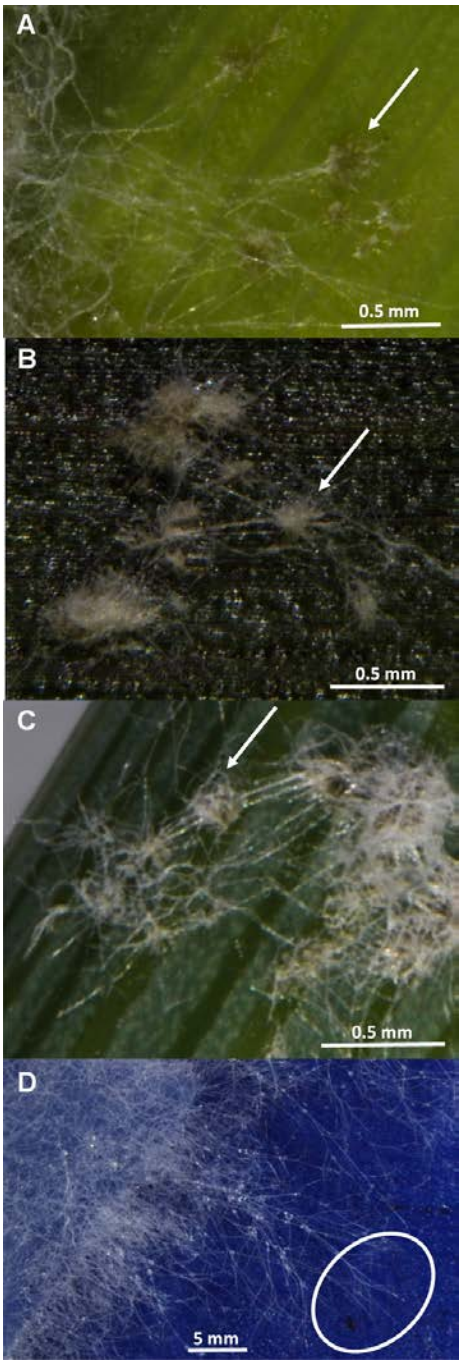

Figure 9: Appearance of *S. sclerotiorum* on leaf and control surfaces 24 hours after inoculation at 22 ^{0}C. **A**, infection cushions on *Acorus gramineus* (30x); **B**, infection cushions on *Pennisetum glaucum* (30x); **C**, infection cushions on *Juncus inflexus* (20x); **D**, Unstructured mycleia on blue construction paper (10x).

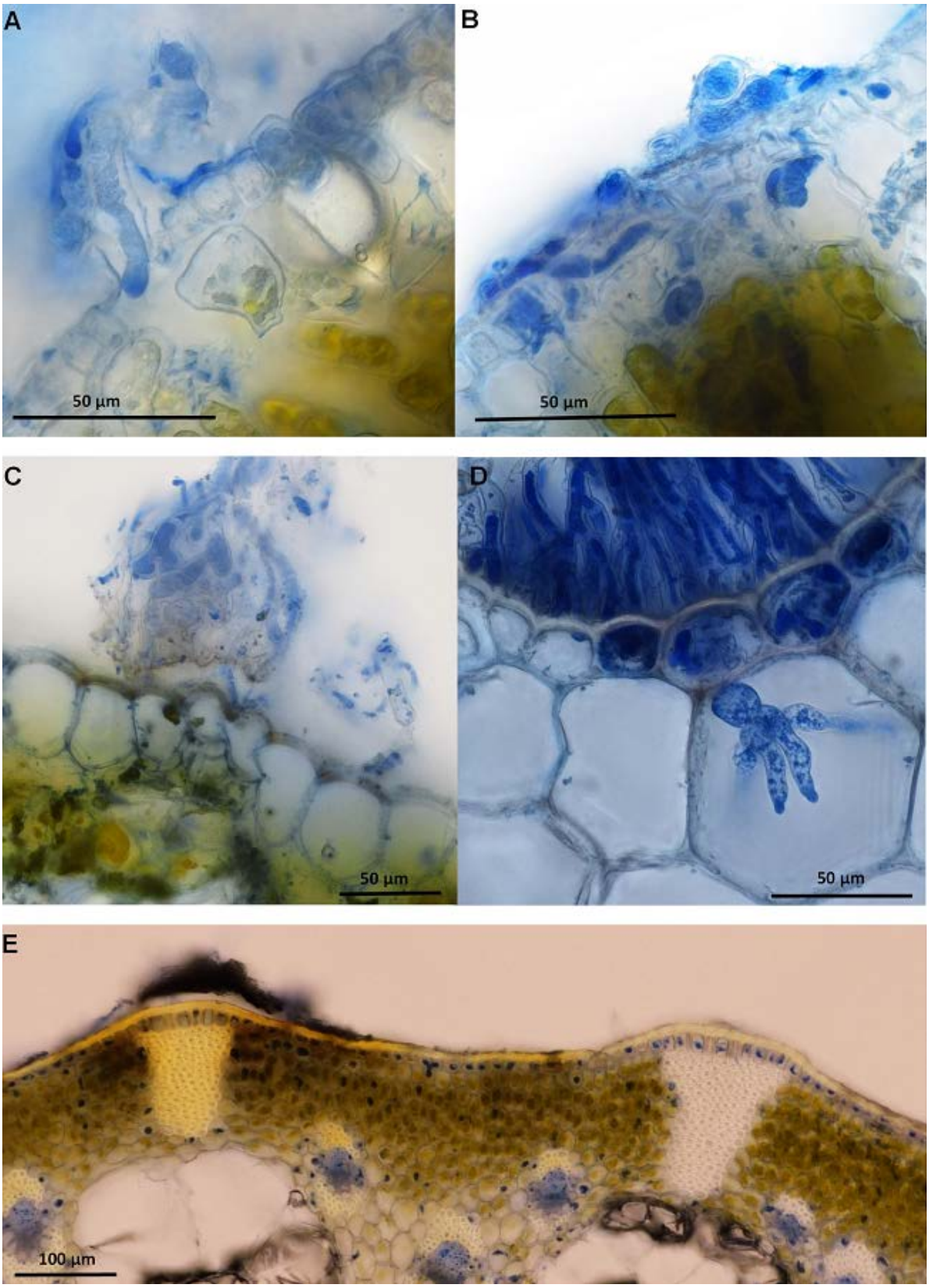
A
50 µm
B
50 µm
C
50 µm
D
50 µm
E
100 µm

Figure 10: A, Hyphae of *S. sclerotiorum* penetrating the epidermis of *Acorus gramineus* 24 hours after inoculation (40x); **B**, *S. sclerotiorum* colonizing epidermal and mesophyll cells of *Acorus gramineus* 24 hours after inoculation (40x); **C**, infection cushion on *Pennisetum glaucum* 24 hours after inoculation (20x); **D**, infection cushion and colonization of epidermal and mesophyll cells of *Pennisetum glaucum* 48 hours after inoculation (40x); **E**, degraded infection cushions on *Juncus inflexus* at 96 hours after inoculation, brown discoloration of healthy epidermal cells, mesophyll cells and cuticle at the infection site (10x).

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