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Susceptibility of longleaf pine roots to infection and damage by four root-inhabiting ophiostomatoid fungi

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ABSTRACT

Restoration of longleaf pine-dominated uplands is common on many public and private lands throughout the southeastern United States. The once dominant longleaf pine ecosystem is important to many now-threatened and endangered plant and animal species, and land managers are increasing efforts to reestablish this fire-dependent forest. Unfortunately, tree mortality in longleaf pine has been observed following attempts to re-introduce prescribed fire. Root-inhabiting ophiostomatoid fungi and their insect vectors have invaded roots of symptomatic longleaf pine. Although, the relationship between ophiostomatoid fungi and longleaf pine roots is poorly understood. In order to assess the pathogenicity and virulence of four ophiostomatoid fungi to longleaf pine, trees within two broad age classes (20–30 and 40–60 years) were used for root inoculations during the fall of 2006 and 2007 along with the spring of 2007 and 2008. All fungal species consistently caused resin-filled, discolored lesions on the phloem surface extending to the xylem. The successful inoculation of healthy longleaf pine roots confirms the pathogenicity of *Grosmannia huntii*, *Leptographium procerum*, *Leptographium serpens*, and *Leptographium terebrantis*. *G. huntii* caused the largest lesions, including 22.20 cm^2, 13.37 cm^2, and 9.21 cm^2 larger than *L. procerum*, *L. terebrantis*, and *L. serpens* respectively. In contrast, *L. procerum* caused significantly smaller lesions than all other fungi, including 8.65 cm^2 smaller than *L. terebrantis* and 10.69 cm^2 smaller than *L. serpens*. Restoration efforts of longleaf pine may be affected by fungal root infection in the future. Future studies should focus on the interactions between stress factors associated with longleaf pine to define more clearly the ecological role of root-inhabiting ophiostomatoid fungi in the ecosystem.

1. Introduction

Longleaf pine (*Pinus palustris* Mill.) was once the dominant *Pinus* species on upland sites throughout the southeastern United States. Once occupying 30 million hectares from Virginia, south to Florida, and west to Texas (Frost, 1993), longleaf pine-dominated land has been reduced to a small fraction of its original range (Outcalt and Sheffield, 1996). Longleaf pine is intimately associated with frequent surface fires, which maintain ecosystem structure and function (Gilliam and Platt, 1999). The longleaf pine ecosystem has been considered one of the most biologically diverse forested systems on the planet (Peet and Allard, 1993). There has been a drastic reduction in forest type since European arrival due to wildfire suppression, the introduction of feral hogs (*Sus scrofa domesticus* L.) (Lipscomb, 1989), and inherently slow natural regeneration (Frost, 1993).

The decline in longleaf pine-dominated forests has resulted in habitat loss for many species, including the red-cockaded woodpecker (RCW) (*Picoides borealis* Vieillot), which is reliant on large living trees for nesting (Conner et al., 2001). Recovery of the RCW and other associated species are dependent on the successful restoration of the longleaf pine-dominated uplands (Conner et al., 2001) and has become a significant priority on many private, state, and federal lands throughout its original range (Alavalapati et al., 2002). With long needles, thick, flakey bark, and a well protected bud, longleaf pine is particularly well adapted for frequent fire disturbance events (Landers, 1991) which must be a part of restoration efforts. Fine fuels produced by longleaf pine help to facilitate frequent burns (Platt et al., 1988), which aid in nutrient turnover,

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herbaceous growth, and the proliferation of a diverse assemblage of organisms in the understory (Carter and Foster, 2003). However, restoration efforts have been plagued with a variety of different challenges (Brockway et al., 2005), including a unexplained premature mortality in longleaf pine, especially following the reintroduction of prescribed fire following a period of fire exclusion (Otrosina et al., 2002).

Annual longleaf pine mortality is relatively low (1.9%) (Boyer, 1979; Palik and Pederson, 1996) despite the frequency and severity of disturbance in longleaf-dominated ecosystems. Also, longleaf pine has few serious insect and disease pests, relative to other closely related southeastern pine species (Snow et al., 1990). The southern pine beetle (SPB) (*Dendroctonus frontalis* Zimmerman) is the largest biotic pest in the southeastern United States and causes catastrophic damage in loblolly (*Pinus taeda* L.) and shortleaf (*Pinus echinata* Mill.) pine. However, longleaf pine is known to be incredibly tolerant of SPB infestation (Friedenberg et al., 2007). It is also more tolerant of the prominent root disease pests *Heterobasidion annosum* (Fr.) Bref. (Platt et al., 1965) and *Phytophthora cinnamomi* Rands, compared to other southeastern *Pinus* species (Barnard et al., 1993). Arguably the most damaging disease of longleaf pine is brown spot needle blight (*Mycosphaerella dearnessii* M.E. Barr), causing mortality in young grass-stage seedlings but not mature trees. On older trees, a group of root-inhabiting ophiostomatoid fungi have recently been associated with dying longleaf pines (Otrosina et al., 1999). Ophiostomatoid fungi, including *Leptographium terebrantis* S.J. Barras and T.J. Perry and *Leptographium procerum* (W.B. Kendr.) M.J. Wingf. were isolated from woody longleaf pine roots following burning (Otrosina et al., 2002) as well as in a more recent survey of longleaf pine roots at Fort Benning Military Installation, Georgia (Zanzot, 2009). Evidence suggests ophiostomatoid fungi are widespread in woody roots of longleaf pine; however, their role in the longleaf pine ecosystem in not well established.

Grosmannia species (previously *Ophiostoma* Zipfel et al., 2006) and their anamorphs, *Leptographium* species have been identified as causal agents of conifer diseases around the world (Wingfield et al., 1988). Under some circumstances, ophiostomatoid fungi have been considered primary pathogens of conifer hosts (Cobb, 1988). The best example is black-stain root disease affecting various conifer species of the western United States, caused by *L. wageneri* (W.B. Kendr.) M.J. Wingf. Infection by ophiostomatoid fungi often result in a pitch-filled resinous lesion with the potential to cause severe occlusion of affected tissues (Hessburg and Hansen, 1987). Significant loss of root conductivity has been detected in naturally infected root sections (Joseph et al., 1998). Recently, mortality in loblolly pine has been closely associated with *L. procerum*, *L. terebrantis*, and possibly *Leptographium serpens* (Goidanich) Siemaszko, along with other biotic and abiotic factors (Eckhardt et al., 2007). Loblolly pine decline is hypothesized to be a product of several different stress factors including topography (Eckhardt and Menard, 2008), insect pest damage (Eckhardt et al., 2004a), and fungal infection (Eckhardt et al., 2004b). Roles of each factor, however, have not been conclusively established. Longleaf pine may be experiencing a similar decline syndrome, although results have been less conclusive (Zanzot, 2009). Some in the southeastern United States believe ophiostomatoid fungi act as facultative pathogens in longleaf pine, causing significant damage in only severely stressed trees (Otrosina et al., 2002).

Although the pathogenicity of several southeastern ophiostomatoid fungi has been shown experimentally in young longleaf pine trees and certain fungal species were determined to be more virulent (Matusick and Eckhardt, 2010a), inoculations of large tree roots provide the most accurate measure of pathogenicity and virulence. A large-scale root inoculation experiment incorporating a range of tree ages, during two seasons, was established in 2006 and 2007 in order to determine if ophiostomatoid fungi cause significant root damage in longleaf pine. It is hypothesized that ophiostomatoid fungi can cause significant damage following inoculation of seemingly healthy longleaf pine roots. The secondary objective is to determine the most damaging root-inhabiting ophiostomatoid species (among four). It is hypothesized that ophiostomatoid fungi vary in their effect on longleaf pine roots. Finally, the season of inoculation will be considered, in order to determine when host trees may incur the most severe root damage.

2. Materials and methods

2.1. Field experiment sites

A total of four longleaf pine stands were used in inoculation tests. All stands were located in the eastern gulf coastal plain of Alabama in Conecuh and Covington Counties. Fall inoculations were performed in four longleaf pine stands, 25, 47 (2006), 27, and 54 (2007) years old, located at the Solon Dixon Forestry Center, Alabama (2006) and Conecuh National Forest, Alabama (2007). Identical inoculation tests were conducted on different trees within the same stands during the spring (2007, 2008). All stands have been maintained with frequent prescribed fire. All trees used in inoculations appeared to be healthy with no crown characters suggesting root disease or stress.

2.2. Treatment description and application

Inoculation tests were performed using a single spore isolate, in the anamorphic state, of each of the following four ophiostomatoid fungi: *G. huntii*, *L. procerum*, *L. serpens*, and *L. terebrantis*. All fungal isolates were collected from roots of southern pines exhibiting local tissue damage and deteriorating crowns using methods described in Eckhardt et al. (2007) (Table 1). Fungal identities have been confirmed by Dr. Mike Wingfield at the Forestry and Agricultural Biotechnology Institute, South Africa using morphological and sequence data. Two weeks prior to each inoculation, all isolates were placed on 2% malt extract agar (MEA).

Fall inoculation tests were performed on September 9, 2006 and November 9, 2007, while spring inoculations were conducted on March 27, 2007 and March 19, 2008. In the fall inoculation tests, two control treatments (wound only and wound + sterile MEA) were incorporated with fungal treatments, while the spring inoculation tests included the wound control only. Fungal isolates were paired with one another, resulting in six total fungal isolate pairings. Pairs of fungal isolates were randomly assigned to nine trees within each stand, and each isolate was applied to one lateral root on each tree. In addition, both control treatments were assigned to each sample tree during the fall, while two wound only controls were assigned to each tree in the spring.

Two primary lateral roots on each sample tree were excavated out to approximately 1.5 m from the root collar area. The fungal inoculation was performed approximately 91 cm from the root collar area (Fig. 1). Additionally, one control treatment was randomly assigned to each root and applied to the lateral root approximately 30 cm from the root collar area. All inoculations were administered using a 13 mm diameter cork borer to create a wound and remove bark tissue from the top of the root, exposing the cambial layer. Next, the 10 mm diameter plug of actively growing mycelium was placed against the exposed tissue. The plug of bark tissue, removed from the wound, was then placed over the mycelia plug and duct tape was used to cover the inoculation site (Wright, 1933). The controls were administered in an identical fashion, with either nothing or sterile MEA in place of fungal

Table 1
Fungal isolates used in inoculations of longleaf pine roots.

Fungal species	Isolate no./ATCC accession no.[a]	Collection site	Host source
Grosmannia huntii	LLP-R-02-100/MYA-3311	Fort Benning Military Reservation, Georgia	Longleaf pine root
Leptographium serpens	LOB-R-00-309/MYA-3315	Westervelt Company Land, Alabama	Loblolly pine root
Leptographium terebrantis	LOB-R-00-805/MYA-3316	Talladega National Forest, Oakmulgee Ranger District, Alabama	Loblolly pine root
Leptographium procerum	LOB-R-00-456/MYA-3313	Talladega National Forest, Oakmulgee Ranger District Alabama	Loblolly pine root

[a] Fungal isolates were obtained from trees exhibiting symptoms characteristic of root disease and reside in collections at the Forest Health Dynamics Laboratory, Auburn University, Auburn, AL.

mycelium for the wound and wound + media controls, respectively. All treatment sites were marked with pin flags prior to burying each root.

2.3. Data collection and analysis

Eight weeks after inoculation, the roots were re-excavated paying careful attention not to damage the roots. All inoculated roots were severed from the tree and removed from the ground for measurements. First, the root diameter was measured at each treatment point. The bark was removed surrounding each treatment and the length and width of discolored vascular tissue was recorded. A clear transparent sheet was used to trace the outline of each discolored lesion and a LASICO® (LASICO Co., Los Angeles, CA) planimeter was used to estimate the total surface area (cm^2) for each discolored lesion (Klepzig and Walkinshaw, 2003). Each root was cut transversely through the point of inoculation, and the total depth of discolored sapwood was measured from the center of each inoculation site. Small sections of tissue were removed from areas surrounding each inoculation site and placed on CSMA (MEA containing 800 mg/l of cycloheximide and 200 mg/l of streptomycin sulfate) for identification.

In order to assess differences among control treatments, the difference was calculated for each tree, resulting in the added effect due to media. The additional media effect was subjected to ANOVA within the general linear model (GLM) procedure using SAS statistical software (SAS Institute, 9th ed., Cary, NC). Experiment year and stand age class (20–30) (40–60) were included as blocked factors in the model. Estimate statements, included in the GLM, allowed for testing whether or not the added effect of MEA was statistically larger than zero.

The control lesion measurement was subtracted from the fungal lesion measurement on each root, resulting in the added effect due to each fungal species. The added effect of each fungal species separately was subjected to an ANOVA in the GLM procedure. Included in the model were year (blocked variable), stand age class (blocked variable), and season. Appropriate estimate statements were included to test whether or not the added effect of each fungal species was significantly greater than zero.

The effect of the fungal inoculation on longleaf pine roots was compared using a similar computational method. On each tree, the difference between the two fungal treatments was calculated and then subjected to an ANOVA in the GLM procedure for each response variable. Year (blocked factor), stand age class (blocked factor), season, fungal pairing, and the interaction between season and fungal pairing were included in the model. Estimate statements were included to test whether or not the difference between the fungi was greater than zero, for each host response variable. The analysis represents a direct comparison between all fungal species, using only those trees in which both fungal species were paired. Seasonal effects were tested for each treatment (wound control, *G. huntii*, *L. procerum*, *L. serpens*, *L. terebrantis*) separately.

3. Results

3.1. Post-treatment observations

A clearly discolored, resin-filled lesion was observed surrounding the point of root inoculation on the surface of the phloem, often extending deep into the sapwood with all ophiostomatoid fungi tested on longleaf pine. Tissue occlusion of more than 60% of the cross-sectional sapwood was observed following several inoculations, though measurements were not recorded for every sample. The pitch response was severe, particularly when *G. huntii* and *L. serpens* were present, creating a large fissure extending the length of the discolored lesion (Fig. 2). In contrast, control inoculations resulted in a weak resin response, with light colored tissue surrounding the treatment point. The presence of sterile media in the wounds caused increases in root lesion size when it was included during the fall inoculations. Lesion length, depth, and area were larger in those wounds that received sterile media (Fig. 3). Despite statistical significance, the authors concluded control differences were not biologically significant considering practical significance and sources of measurement error. As a result, control treatments were considered to be equal for further analyses.

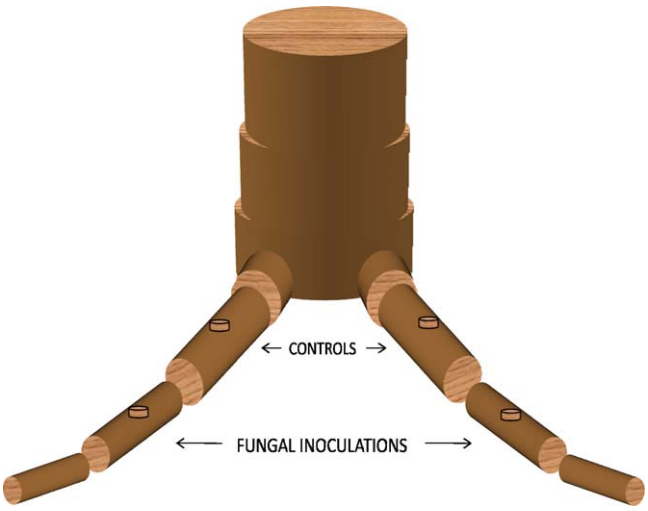

Fig. 1. Treatment distribution on each tree. Control treatments were administered proximal and fungal inoculations were performed distal.

Fig. 2. Large resin-filled fissure extending from the point of inoculation 8 weeks following introduction of *G. huntii*.

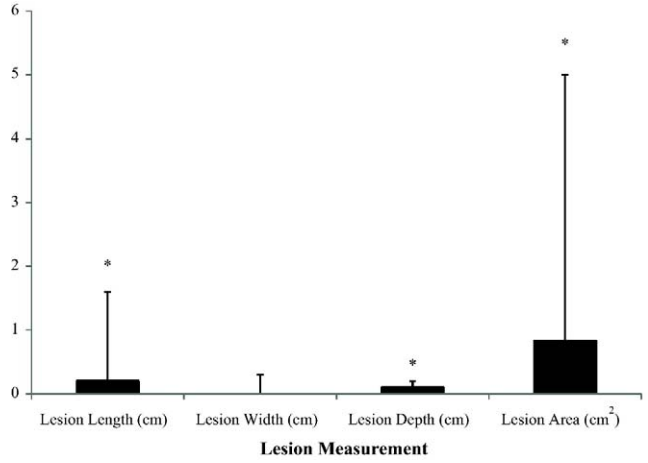

Fig. 3. Average increase in lesion length, width, depth, and area attributed to the presence of sterile media in control wounds. The asterisks denote value, which is statistically greater than zero at alpha = 0.05. Error bars represent the standard deviation of the mean.

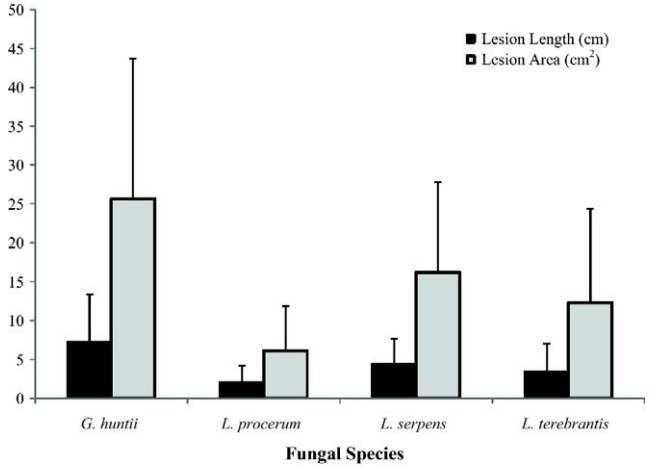

Fig. 5. Lesion length and area attributed to fungal species following subtraction of controls. All comparisons are statistically greater than zero at alpha = 0.05. Error bars represent the standard deviation of the mean.

3.2. Treatment comparisons

Root inoculation with *G. huntii, L. serpens, L. procerum,* and *L. terebrantis* caused wider, deeper (Fig. 4), longer, and larger (cm^2) (Fig. 5) lesions compared to controls. *G. huntii* caused the greatest lesion length, lesion width, and total lesion area when compared to all other fungal species tested (Table 2). *L. serpens* inoculation resulted in larger, ($T = 2.32$, $P = 0.0209$) but not longer ($T = 1.56$, $P = 0.1202$) lesions compared to *L. terebrantis*. All fungal species caused longer and larger lesions than *L. procerum. G. huntii* resulted in a deeper lesion than *L. serpens* ($T = 5.26$, $P < 0.0001$) and *L. procerum* ($T = 9.28$, $P < 0.0001$), but not *L. terebrantis* ($T = 1.22$, $P = 0.2247$). *L. terebrantis* caused deeper lesions than *L. serpens* ($T = -2.82$, $P = 0.0050$). *L. procerum* consistently produced lesions shallower than all other species. Root infection was confirmed by re-isolating the fungi. Successful re-isolation from *G. huntii*, *L. procerum*, *L. serpens*, and *L. terebrantis* was 70%, 73%, 69%, and 71% respectively.

3.3. Seasonal effects on lesion development

The season in which inoculations were conducted significantly affected lesion development. The mean lesion depth was larger during the spring inoculations when *G. huntii*, *L. terebrantis*, and *L. procerum* were used (Fig. 6). However, the wound control had deeper lesions in the fall, but the overall size was not different between seasons (Fig. 7). Lesion area was larger during the spring inoculations for each fungal treatment tested on longleaf pine.

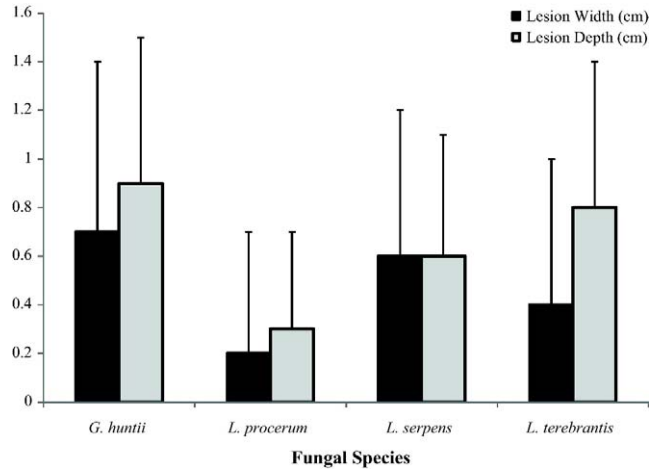

Fig. 4. Lesion width and depth attributed to fungal species following subtraction of controls. All comparisons are statistically greater than zero at alpha = 0.05. Error bars represent the standard deviation of the mean.

4. Discussion

These studies were designed to represent a conclusive test of tissue pathogenicity, including trees from several different age classes, stands, and seasons. Each fungal species infected inoculated roots and affected more tissue than controls, confirming their pathogenicity to longleaf pine roots. Similar conclusions have been made previously with respect to *L. serpens* (Matusick et al., 2008), *G. huntii*, *L. procerum*, and *L. terebrantis* in longleaf pine seedlings (Matusick and Eckhardt, 2010b). In addition, Otrosina et al. (2002) observed *L. procerum* and *L. terebrantis* causing significant lesion development in roots and stems of longleaf pine.

A darkened lesion is commonly observed in conifer hosts around the world following inoculation with ophiostomatoid fungi (Kuroda, 2005; Plattner et al., 2008; Solheim et al., 2001). Increases in oleoresin flow are characteristic in tissues surrounding artificial inoculations (Klepzig et al., 2005; Knebel et al., 2008), particularly following the introduction of highly pathogenic species (Cobb, 1988). Lesions form on the surface of the phloem, often extending deep into the xylem parenchyma (Nagy et al., 2005). Identical observations were made following our inoculations of longleaf pine roots and are consistent with inoculation of *Pinus* hosts with other pathogenic ophiostomatoid fungi (Lee et al., 2006; Solheim and Krokene, 1998). In contrast, control inoculations with sterile agar and control wounds initiate a passive desiccation of damaged tissues immediately surrounding the inoculation point, with little or no lasting damage of conducting tissues (Klepzig and Walkinshaw, 2003).

Lesions following inoculation with ophiostomatoid fungi are known to increase with increasing fungal virulence (Owen et al., 1987; Parmeter et al., 1989). It is thought that ethylene production triggers an increase in monoterpene synthesis, which regulates the size of the resulting lesions (Popp et al., 1995). Of the fungi tested, *G. huntii* was the most virulent on longleaf pine roots. These tests are the first to confirm the pathogenicity and virulence of *G. huntii* on large longleaf pine. *L. serpens* was also more virulent than both *L. terebrantis* and *L. procerum* isolates. These findings support previous reports in loblolly pine, which found *L. serpens* to cause larger

Table 2
Average treatment residual (A-B) and probability of greater *F*-statistic for lesion length, width, depth, and area.

Treatment comparison A–B	*N*	Lesion length[a] (cm)	*P*-value	Lesion width (cm)	*P*-value	Lesion depth (cm)	*P*-value	Lesion area (cm^2)	*P*-value
G. huntii–L. serpens	70	3.0 (6.1)	0.0001	0.3 (0.7)	0.0001	0.4 (0.6)	0.0001	9.21 (16.25)	0.0001
G. huntii–L. terebrantis	72	3.2 (4.0)	0.0001	0.4 (0.7)	0.0001	0.1 (0.7)	0.2297	13.37 (16.42)	0.0001
G. huntii–L. procerum	73	6.3 (11.9)	0.0001	0.4 (0.7)	0.0001	0.6 (0.6)	0.0001	22.20 (23.02)	0.0001
L. serpens–L. terebrantis	71	1.1 (4.5)	0.1202	0.2 (0.8)	0.0100	−0.2 (0.6)	0.0050	4.27 (12.67)	0.0209
L. serpens–L. procerum	72	3.0 (3.7)	0.0001	0.5 (0.5)	0.0001	0.3 (0.4)	0.0001	10.69 (11.29)	0.0001
L. terebrantis–L. procerum	69	2.4 (3.2)	0.0014	0.3 (0.6)	0.0001	0.5 (0.7)	0.0001	8.65 (11.32)	0.0001

[a] Means (followed by standard deviation in parentheses) with *P*-value ≤0.05 are significantly different from zero.

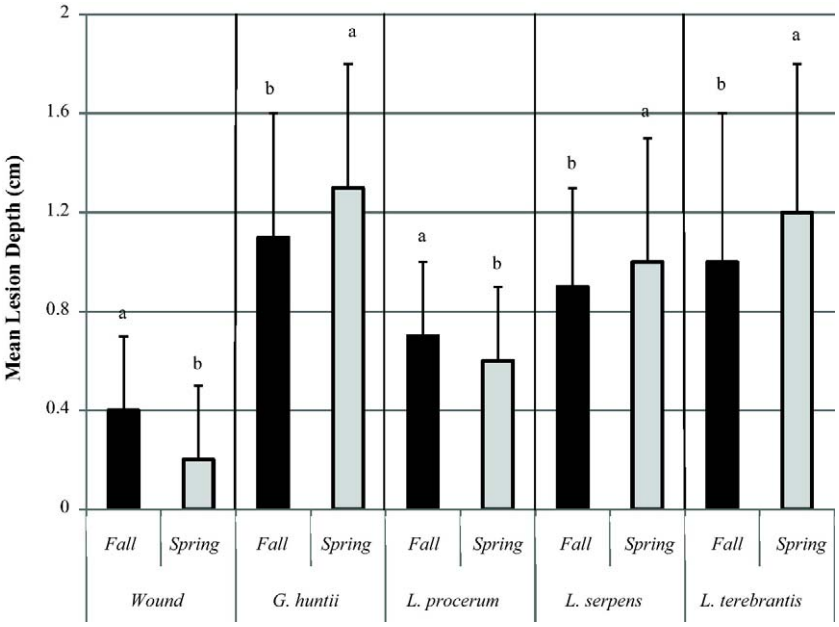

Fig. 6. Average lesion depth of discolored lesions following wound inoculation with *G. huntii*, *L. procerum*, *L. serpens*, *L. terebrantis*, and wound control in fall and spring tests. Letters denote seasonal comparisons at alpha = 0.05 for each treatment separately. Error bars represent standard deviation of the mean.

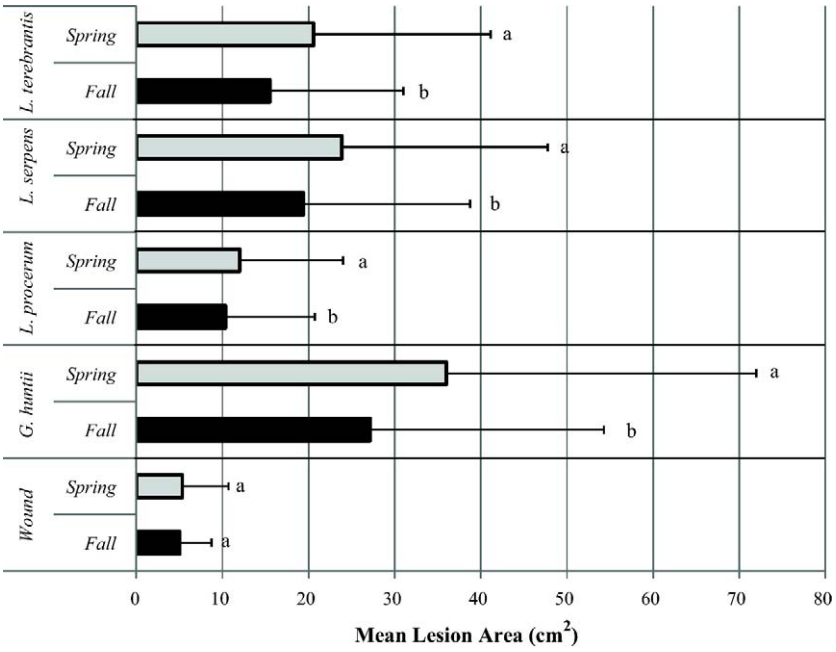

Fig. 7. Average discolored lesion surface area following wound inoculation with *G. huntii*, *L. procerum*, *L. serpens*, *L. terebrantis*, and wound control in fall and spring tests. The length of each bar represents the mean lesion area following each treatment prior to subtraction of controls. Letters denote seasonal comparisons at alpha = 0.05 for each treatment separately. Error bars represent standard deviation of the mean.

lesions in seedlings and large tree stems (Eckhardt et al., 2004b). *L. serpens* has been associated with disease of *P. pinaster* Aiton. and *P. radiata* D. Don. in South Africa. In root inoculation tests, *L. serpens* produced lesions extending 20 cm after 6 months in *P. pinaster* and *P. radiata* (Wingfield and Knox-Davies, 1980). Discoloration associated with *G. huntii* and *L. serpens* inoculation extended far from the inoculation point on average and penetrated deep into susceptible sapwood, resulting in damage to host tissues. Although no clear host physiological disruption was detected, severe occlusion of roots does lead to a reduction in hydraulic conductivity (Joseph et al., 1998). *L. terebrantis* also caused larger lesion areas than *L. procerum* in our study, which is in line with findings that *L. terebrantis* is more pathogenic than *L. procerum* (Eckhardt et al., 2004b; Klepzig and Walkinshaw, 2003; Klepzig et al., 1996; Wingfield, 1986). *L. procerum* was the least damaging species tested but is the species most commonly isolated from longleaf pine (Zanzot, 2009).

G. huntii is more virulent than all other fungal species tested; however, like all fungi used in these studies, transmission of *G. huntii* to longleaf pine roots is dependent on the actions of its vectors. *G. huntii* is closely associated with a variety of bark beetle vectors around the world (Jacobs and Wingfield, 2001) including root-feeding bark beetles *Hylastes tenuis* Eichhoff and *Hylastes salebrosus* Eichhoff in longleaf pine stands (Zanzot et al., 2010). Despite the association between *G. huntii* and root-feeding bark beetles, longleaf pine roots naturally infected by *G. huntii* have been rarely detected (Zanzot, 2009). However, results from these findings could be misleading, due to the systematic sampling of longleaf pine root tissue, with most samples taken from healthy root sections (Matusick observations). *L. terebrantis* and *L. procerum* are commonly isolated from root tissue using this method (Zanzot, 2009). However, *G. huntii* causes a distinct set of severe local symptoms and it is likely that sampling of damaged, occluded roots would more efficiently detect its presence.

Several studies have illustrated seasonal patterns of lesion size and development following inoculation with ophiostomatoid fungi (Horntvedt, 1988; Paine, 1984; Reid and Shrimpton, 1971). Larger lesion sizes are routinely observed during the growing season when compared to the dormant months (Stephen and Paine, 1985). Lesion development is partially a product of starch conversion to resin constituents for defense, which decreases continuously from May to December in loblolly pine (Blanche et al., 1992). Cook et al. (1986) found a clear relationship between lesion size and temperature, with the largest lesions formed during the summer months, decreasing into the fall. Additional support for this hypothesis is that root cells formed shortly following differentiation from the meristem have the greatest potential for xylem resin production (Beryman, 1972). These longleaf pine studies illustrate, larger, and with certain fungal species, deeper lesions during the spring ("growing") season. Some authors have suggested lesion area following inoculation of host stems represents the tree's ability to defend itself against invading bark beetles and pathogens (Paine et al., 1997). However, following inoculation of host roots, it is clear that significant sapwood damage results from the lesion response, and may lead to significant losses of hydraulic conductivity resulting from tissue occlusion, particularly during periods of strong response. Joseph et al. (1998) observed loss of conductivity in roots naturally affected by black-stain root disease and similar findings have been shown experimentally following inoculation of host stems (Kuroda, 2005; Matusick et al., 2008).

On the ecosystem scale, the consequences of infection by root-inhabiting ophiostomatoid fungi in longleaf pine roots are poorly understood. It is not likely that longleaf pine mortality can be attributed to root fungal infection alone. More realistically, root infection by ophiostomatoid fungi should be considered among the group of stress factors that are regularly experienced by longleaf pine. For example, the re-introduction of cyclical burning regimes has occurred throughout many areas of southeastern United States and is known to increase tree stress (Varner et al., 2005). As a result, common bark beetle and weevil vectors are attracted to stands following severe burns (Sullivan et al., 2003) and introduce root-inhabiting ophiostomatoid fungi (Hanula et al., 2002). Otrosina and Ferrell (1995) suggest common secondary pests (i.e. bark beetles, and root-inhabiting ophiostomatoid fungi) can act as primary pests when stand disturbance regimes are altered. There is clearly the potential for root damage in longleaf pine by ophiostomatoid fungi and restoration of longleaf pine-dominated uplands may be hampered by root disease associated with ophiostomatoid fungi in the future. Further studies on the interactions among stress factors will more clearly define the role of root-inhabiting ophiostomatoid fungi in longleaf pine mortality.

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