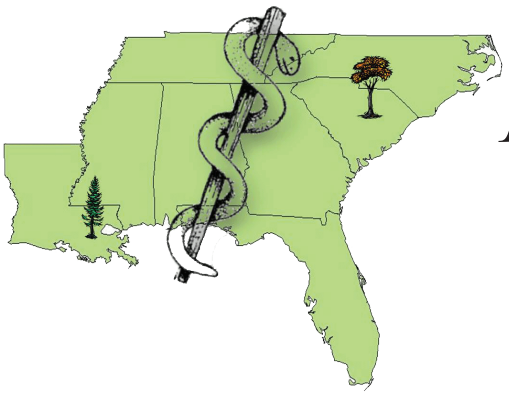

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VARIATION IN VIRULENCE AMONG FOUR ROOT-INHABITING OPHIOSTOMATOID FUNGI ON *PINUS TAEDA L.*, *P. PALUSTRIS MILL.*, AND *P. ELLIOTTII ENGELM.* SEEDLINGS

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ABSTRACT
Ophiostomatoid fungi have been recently implicated in root disease of pines in the southeastern United States. To determine their virulence, inoculation studies were conducted on loblolly (*Pinus taeda*), longleaf (*Pinus palustris*), and slash pine (*Pinus elliottii*). One year-old bareroot seedlings were wound-inoculated with one of four prominent North American ophiostomatoid fungal species. After three months, a darkened lesion, extending from the point of inoculation was observed for all species. *Grosmannia huntii* (*L. huntii*) caused the greatest lesion and occlusion length in loblolly pine and slash pine. *Leptographium procerum* and *L. terebrantis* caused similar lesion and occlusion lengths and were the smallest among the fungal treatments. These studies indicate clear virulence differences among the four North American fungi. *Grosmannia huntii*, previously not known to be pathogenic, caused significant damage compared to other well-known *Leptographium* species. Finally, lesion and tissue occlusion lengths were significantly smaller in longleaf pine for all fungal species when compared to loblolly and slash pine.

INTRODUCTION
Root-inhabiting ophiostomatoid fungi have caused disease in many pine systems throughout the world (Wingfield et al. 1988). In some instances, ophiostomatoid fungi act as primary pathogens, causing mortality to their host. *Leptographium wageneri* (W.B. Kendr.) M.J. Wingf, a virulent primary pathogen, causes extensive pine mortality throughout the northwestern United States (Cobb 1988). In other cases, ophiostomatoid fungi may only act as stressors, in a larger complex, that ultimately leads to tree mortality (Otrosina et al. 2002). Root-inhabiting ophiostomatoid fungi have been identified as contributors to pine decline in several systems, including red pine (*Pinus resinosa* Ait) in the great lake states (Klepzig et al. 1991), eastern white pine (*P. strobus* L.) in the northeastern United States (Dochinger 1967) and most recently, loblolly pine (*P. taeda* L.) in the southeastern United States (Eckhardt et al. 2007).

Several ophiostomatoid fungi with *Leptographium* anamorphs, including *L. procerum* (Kendrick) M.J.

Wingfield, *L. terebrantis* S.J. Barras & T.J. Perry, *L. serpens* (Goidanich) Siemaszko, and *Grosmannia huntii* (R.C. Rob. Jeffr.) Zipfel, Z.W. de Beer & M.J. Wingf. (*L. huntii* M.J. Wingfield) have been isolated from the roots declining pine throughout the southeastern United States, including Georgia (Menard et al. 2006), Alabama (Eckhardt et al. 2007), and South Carolina (Otrosina et al. 2002).

Leptographium procerum, *L. terebrantis*, and *L. serpens* have been consistently associated with symptomatic loblolly pine (Eckhardt et al. 2007). Each *Leptographium* species has been isolated from longleaf pine (*P. palustris* Mill.) root tissue (Zanzot 2009). *Grosmannia huntii* (formerly *Ophiostoma huntii* [Zipfel et al. 2006]) has recently been isolated from loblolly root tissue and bark beetles breeding in pine hosts (Matusick and Eckhardt unpublished data). While root disease and decline associated with *Leptographium* species have not been well documented in slash pine (*P. elliottii* Engelm.), *L. procerum* has been isolated from stump (Barnard et al. 1991) and root tissues (Horner and Alexander 1983).

Leptographium procerum and *L. terebrantis* are two well-known ophiostomatoid fungal root pathogens of North American pines (Wingfield et al. 1988). *Leptographium procerum* is the causal agent of procerum root disease in eastern white pine (Dochinger 1967) and is associated with various conifer species around the world (Jacobs and Wingfield 2001), particularly *Pinus* species within the United States (Alexander et al. 1988). In inoculation studies, *L. procerum* has been reported to be weakly virulent (Wingfield 1983). *Leptographium terebrantis* is only found in North America and has been associated with various diseases of pine (Jacobs and Wingfield 2001).

Leptographium terebrantis consistently causes resin-soaking (Nevill et al. 1995), sapwood discoloration (Rane and Tattar 1987), and long vertical lesions in *Pinus* hosts (Wingfield 1986). *Leptographium terebrantis* is considered a moderate to severe pathogen, often causing mortality (Harrington and Cobb 1983). In loblolly pine seedling inoculations, Eckhardt et al. (2004a) found *L. procerum* to readily infect root tips and cause root and foliar dieback. In the same study, *L. terebrantis* caused darkly stained lesions measuring 20 mm after four months.

Leptographium serpens and *G. huntii* have been less commonly reported in North American pines. However, Eckhardt et al. (2007) isolated *L. serpens* from loblolly pine where it was associated with decline symptoms and root-feeding insect vectors. *Grosmannia huntii* has previously been found invading *Pinus* hosts of more northern latitudes in North America (Olchowecki and Reid 1974) and has been closely associated with damage caused by insects. *Hylastes porculus* Erichson vectors the fungus in red pine stands (Klepzig et al. 1991) and mountain pine beetle (*Dendroctonus ponderosae* Hopk.) in lodgepole pine (*P. contorta* Douglas var. *latifolia* Engelmann) (Solheim 1995). Despite some evidence that *L. serpens* is moderately to severely virulent to *Pinus* species (Eckhardt et al. 2004a), some consider *L. serpens* to be a weak pathogen in South Africa (Zhou et al. 2002). Unlike other *Leptographium* species, no information is available pertaining to the virulence of *G. huntii*.

Past studies with *Leptographium* species in the southeastern United States have mainly focused on inoculations with *L. procerum* and *L. terebrantis* on loblolly (Lackner and Alexander 1981a) and longleaf (Otrosina et al. 2002) pine. More recently, *L. serpens* has been included in inoculations of loblolly pine (Eckhardt et al. 2004a). However, the relative virulence of

ophiostomatoid fungi in the southeastern United States is not known. These studies were initiated in order to test the hypothesis that root-inhabiting ophiostomatoid fungi are equally virulent to southern pine hosts.

MATERIALS AND METHODS

Isolates of *L. procerum*, *L. terebrantis*, *L. serpens*, and *G. huntii* were obtained from either loblolly or longleaf pine roots (Table 2.1) exhibiting decline disease symptoms. Primary lateral roots were excavated and tissues were obtained using methods described in Eckhardt et al. (2007). All isolates used in the inoculation tests were in the anamorphic state, were from single-spore isolations and have been used in other studies (Eckhardt et al. 2004a, 2008).

Bareroot seedlings of loblolly pine, slash pine and longleaf pine were obtained from the Smurfit Stone Rock Creek Nursery near Brewton, Alabama. A total of 250 bareroot seedlings of each species were planted with ProMix BX® (Premier Tech, Quebec, Canada) peat-based potting mix in one-gallon plastic pots one week following lifting in December 2007. A two-factor experiment, including three pine hosts and the four fungal species, was housed in an outdoor screen facility on the Auburn University campus. The building environment was homogeneous, unobstructed from sunlight and accessible to natural precipitation. After eleven weeks, prior to imposing the inoculation treatment, dead trees were removed from each group leaving a total 225 seedlings of each pine species respectively to be used in the experiment. In December of 2008, the study was repeated using a total of 225 seedlings per species (or 200 seedlings after culling).

The four fungal treatments and an unwounded control were randomly assigned to an equal number of seedlings. Each fungal isolate was placed on 2% malt extract agar (MEA) two weeks prior to inoculation. Seedlings assigned to the four fungal treatments were wound inoculated in the lower stem approximately 2 cm from the soil line. A small (1 cm) vertical slit was made with a sterile razor blade extending into the vascular tissues, followed by placing a 3 mm diameter plug of colonized MEA in the wound (Fig. 2.1). The inoculation was wrapped in moist cotton and sealed with Parafilm®, as described by Eckhardt et al. (2004a).

At the culmination of the study, a subset of five seedlings was randomly selected from each treatment x pine group for pine needle water potential measurements. Measurements were made at predawn and midday on one seedling from each group for five straight days in 2007 using a pressure chamber (Model 670, PMS Instrument Inc. Albany, OR). In 2008 measurements were made over a 3 day period. Two fascicles from the first flush of the current year on each plant were measured, and then averaged to obtain one value for each tree at both predawn and midday.

Twelve weeks after inoculation, seedlings were destructively sampled and the final root collar diameter was measured. The lateral and fine roots were removed from the seedlings and the biomass was weighed after drying for 3 days at 70° C. The stem and taproot biomass was determined after lesion and occluded tissue measurements. Living seedlings were inspected for the presence of cambial lesions and the lesion length and length of occluded (blocked) vascular tissue was measured. Lesion length was considered the total length of darkly pigmented tissue and may or may not have extended the total length of the wound. To determine the length of occluded tissue, the living shoot was placed in a FastGreen stain (FastGreen FCF; Sigma

Chemical Co.) and water solution (0.25 g/liter) (adapted from Nevill et. al. [1995]). After three days, the length of stem tissue not stained by solution was recorded. A 1 cm segment of stem tissue at the lesion margin was removed and placed on CSMA (MEA containing 800 mg/l of cycloheximide and 200 mg/l of streptomycin sulfate) to confirm the fungal infection in inoculated trees.

Seedling host response variables were analyzed using a general linear model (GLM) in SAS statistical software (SAS Institute, 9th ed., Cary, NC). All binary response variables, including survival, lesion presence and re-isolation of fungal species were transformed to percentages for each treatment x pine species combination. All continuous response variables, including root collar diameter, stem and fine root biomass, lesion length and occlusion length were analyzed using the seedling as the experimental unit. Stem and fine root biomass values were transformed using the square-root function to ensure a normal distribution. In the model, each experiment (2007, 2008) was considered a replicate (blocked factor). Both testable factors, including pine and fungal species as well as their interaction were included in the linear model. All pair-wise comparisons were analyzed using Tukey's multiple comparison test, followed by contrast statements. When testing water potential measurements, the two years were combined and the day of measurement was considered blocked. Treatment and tree main effects along with their interaction were included as testable factors.

RESULTS

Within a given pine species, the assigned treatments varied in ther effect on RCD and seedling stem biomass (Table 2.2). Tree species and treatment independently affected the biomass of the fine root tissue. Seedlings inoculated with *G. huntii* had the smallest mean RCD among the treatments in longleaf pine; however, the same affect was not observed in other pines (Table 2.3). Seedlings inoculated with *L. serpens* resulted in slightly more stem mass compared with those inoculated with *G. huntii*. The same difference was not observed in slash and loblolly pines. Longleaf pine seedlings had the greatest lateral and fine root biomass among the tree species (Table 2.4) and seedlings assigned to the *L. procerum* treatment had the highest biomass among the treatments (Table 2.5).

Mortality was observed throughout each experiment in all pine species. Seedling survival was significantly different between pine species (F=15.73, P=0.0003) (Table 2.6). Slash and longleaf pine had less seedling survival when compared to loblolly pine (Table 2.7). Inoculation with fungi did not affect seedling survival (F=0.53, P=0.7177).

All fungal species caused dark sunken or sometimes slightly raised lesions in all pine species tested (Fig. 2.2). Lesions extended vertically from the wounded area, with little evidence of radial movement. Callus tissue was associated with fungal inoculation, most notably in seedlings treated with *L. procerum* and *L. terebrantis* (Fig. 2.3). Within each pine species, the presence of lesions was different among fungal species (F=5.66, P=0.0066). Lesions were detected in nearly all pines inoculated. However in longleaf pine, *L. procerum* and *L. terebrantis* caused lesions in 80 and 85 percent of seedlings respectively, fewer than other treatment x host combinations.

The average lesion length varied among fungal species, within a given pine host (F=8.53, P<0.0001). Lesion development following fungal inoculation was poorest in longleaf pine for

each species. The inoculation of *G. huntii* into pine seedlings resulted in the largest average lesion lengths, though not different from *L. serpens* in longleaf pine (F=0.10, P= 0.7572). Seedling lesions from *L. procerum* and *L. terebrantis* inoculation were the smallest among *Leptographium* species treated but not different from each other on loblolly and slash pine. Lesions found on *L. terebrantis* inoculations were longer than
L. procerum in longleaf pine.

Similar to lesion length, *G. huntii* caused the largest occlusions in all pine hosts treated. *Leptographium serpens* caused the second greatest tissue occlusion in each of the pine species. In all pine species, *L. procerum* caused the smallest average tissue occlusion, often not significantly different from *L. terebrantis*. Occlusion length was smallest for each fungal species in longleaf pine.

All fungal species were successfully re-isolated from the inoculated pines with re- isolation percentage affected by the pine host (F=10.52, P=0.0028). Re-isolation was less successful in longleaf pine seedlings when compared to loblolly and slash pines. No differences were observed in re-isolation among the four fungal species (F=2.77, P=0.0920).

Predawn and midday water potential measurements were not affected by fungal treatment but were different among tree species used (Table 2.8). Longleaf pine seedlings had significantly higher predawn and midday water potential measurements compared to loblolly and slash pine seedlings (Table 2.9).

DISCUSSION

All ophiostomatoid species tested were capable of successful infection and development of local symptoms in southern pine seedlings; however variation between fungal pathogens within certain hosts existed. *Grosmannia huntii* caused the longest lesions and occluded tissue in loblolly pine slash pines. These studies are first to confirm lesion development and damage following artificial inoculation with *G. huntii*. Lesions following inoculation with *L. terebrantis* and *L. procerum* were consistently smaller than other treatments.

An unwounded control was chosen based primarily on results of previous studies concerning many of the same ophiostomatoid fungi. In pine seedlings, it is well established that wounding without the introduction of fungi results in only callus tissue, which encloses the wound without formation of a resinous lesion (Eckhardt et al. 2004a; Klepzig et al. 1995; Wingfield 1986). The wounding method used, causes minor, temporary damage and does not contribute to an increased mortality (Chapter 3). In addition, the pathogenicity of many of the same ophiostomatoid fungi has been established previously (Eckhardt et al. 2004a; Nevill et al. 1995). These tests were initiated to test virulence differences among the four ophiostomatoid fungi, with little interest in testing their ability to infect and produce lesions, compared to wounded controls.

Lesions were readily observed surrounding the point of inoculation in seedlings, with few exceptions. Discolored lesions often appeared sunken and commonly extended beyond the wound beneath the surface of the epidermis. Callus tissue (wound periderm) was present in loblolly pine and slash pine seedlings surrounding the wounded site only, clearly not extending to the lesion margin. Similar lesion morphology and occurrence have been observed in previous

seedling inoculation studies with many of the same fungal species (Eckhardt et al. 2004a). Lesion occurrence was overall lower in longleaf pine seedlings inoculated with *L. procerum* and *L. terebrantis*. It was apparent that under some circumstances lesions failed to develop following inoculation. Longleaf pine is known to be more resistant to many other insect and disease pests (Snow et al. 1990), but these studies are the first to illustrate resistance to ophiostomatoid fungi. Longleaf pine resin has been shown to inhibit growth of ophiostomatoid fungi in vitro, particularly *L. procerum* (Eckhardt et al. 2008).

Lesions associated with fungal infection extended above and below the point of inoculation. Lesions were primarily oriented longitudinally with poor evidence of radial movement. Radial movement is most characteristic of highly virulent ophiostomatoid fungi such as *L. wageneri* (Cobb 1988) and in some instances *L. terebrantis* (Wingfield 1983). Lesions associated with *G. huntii* were larger than *L. serpens* in loblolly and slash pine seedlings. However, *G. huntii* and *L. serpens* infection were not significantly different in longleaf pine. *Leptographium serpens* has been previously observed causing mortality in *Pinus* species as well as similarly large lesions in controlled experiments (Wingfield and Knox-Davies 1980). These inoculation studies represent the first report of *G. huntii* causing significant damage in pine tissue, following artificial inoculation. *Grosmannia huntii* is known as a proficient sapstainer in large pine trees and logs (Robinson-Jeffrey and Grenchenko 1964; Kim et al. 2005b); however it has not been shown to be pathogenic. Lesions following *L. terebrantis* and *L. procerum* inoculation were consistently smaller than those formed from *G. huntii* and *L. serpens*. In similar studies with loblolly pine seedlings, *L. terebrantis* was shown to cause larger lesions than *L. procerum* (Eckhardt et al. 2004a; Nevill et al. 1995). Previous studies have found smaller lesions associated with *L. procerum* when compared to other *Leptographium* species (Wingfield 1983). These new inoculation trials support previous findings that consider *L. procerum* a mild pathogen to *Pinus* species (Harrington and Cobb 1983).

Tissue occlusion is often observed associated with invasion by root-inhabiting ophiostomatoid fungal species (Wingfield and Knox-Davies 1980). Occlusion of vascular tissue has been detected in the past as a measure of host response to infection (Nevill et al. 1995). Generally, occlusion length closely mirrors measures of lesion length and gives supportive evidence to the virulence of ophiostomatoid species (Eckhardt et al. 2004a). Occlusion length was greatest in loblolly and slash pine seedlings infected by *G. huntii*. Tissue occlusion was smaller in seedlings inoculated with *L. terebrantis* and *L. procerum*, when compared to *G. huntii* and *L. serpens*. Similar trends were observed by Eckhardt et al. (2004a) when determining the occlusion of several of the same *Leptographium* species to loblolly pine. Comparable occlusion lengths were observed in a previous experiment with *L. serpens* inoculations of longleaf pine seedlings (Chapter 3).

Consistent re-isolation of inoculated ophiostomatoid species confirms the ability to infect and grow within pine host tissue. However, re-isolation of fungal species from longleaf pine seedlings was statistically lower compared to loblolly and slash pine. Longleaf pine is resistant to several insect and disease pests (Snow et al. 1990), including root disease (Hodges 1969). Recent observations confirm that growth of *Leptographium* species are negatively affected by constitutive longleaf pine resin (Eckhardt et al. 2008). Fungal growth was least affected by loblolly pine resin and more impeded when in the presence of longleaf pine resin. The virulence

data coupled with previous findings suggest that longleaf pine resin may restrict movement within longleaf pine tissue, making it more resistant of ophiostomatoid fungal infection and growth.

Despite development of local symptomology, infection and tissue damage as a result of fungal infection did not result in conclusive variation in whole-tree symptomology. In living seedlings, root collar diameter and stem + taproot biomass was relatively consistent across treatments. Although longleaf pine seedlings inoculated with *G. huntii* were found to have a slightly smaller mean RCD, significant variation was observed between the seedling stock used. Since no pretreatment RCD measurements were taken, the slightly smaller average RCD cannot be fully attributed to *G. huntii*. In addition, inoculation with fungi had no significant effect on seedling water potential, suggesting damage was not causing increased tension in the vascular column. The lack of whole-tree symptomology in inoculated trees resulted in mortality values similar to controls. It is probable that if the experiment were longer in duration or more inoculations were imposed on each seedling, whole-tree symptomology would have been detected. Rane and Tattar (1987) found significant losses of xylem pressure potential 15 days following inoculation of *L. terebrantis*. However, two inoculations were performed on either site of the stem, in contrast to just a single inoculation in this study. In addition, previous experiments that have noted significant treatment mortality were left for a longer duration (Harrington and Cobb 1983; Nevill et al. 1995; Wingfield 1983). Seedling measurements were larger for longleaf pine seedlings compared to loblolly and slash pine. These differences can be fully attributed to the unique phenology and growth habits of longleaf pine seedlings.

Grosmannia huntii produced larger lesion and occlusion lengths in loblolly and slash pine seedlings. In contrast, *L. procerum* and *L. terebrantis* caused the smallest average lesion lengths in loblolly and slash pine. Infection and virulence in longleaf pine seedlings were less apparent, when compared to other southern *Pinus* hosts. Despite smaller lesions, and in some cases, poorer infection in longleaf pine, ophiostomatoid fungi are capable of causing local symptomology similar to that observed in loblolly and slash pines. Disease symptomology was restricted to areas surrounding the infection point with poor evidence of significant xylem dysfunction and foliar symptoms after three months. Future studies concerning root-inhabiting ophiostomatoid species and southern pine mortality should focus on large, mature trees and the role *G. huntii* plays in relation to the other more commonly published species.

Table 2.1 Fungal isolates used in pine seedling inoculation experiment.

Fungal Species	Isolate no./ ATCC accession no.	Collection Site	Host Source
G. huntii	LLP-R-02-100/ MYA-3311	Fort Benning Military Reservation, GA	Longleaf Pine Root
L. serpens	LOB-R-00-309/ MYA-3315	Westervelt Company Land, AL	Loblolly Pine Root
L. terebrantis	LOB-R-00-805/ MYA-3316	Talladega National Forest, Oakmulgee Ranger District, AL	Loblolly Pine Root
L. procerum	LOB-R-00-456/ MYA-3313	Talladega National Forest, Oakmulgee Ranger District, AL	Loblolly Pine Root

Note: All fungal isolates were obtained from trees exhibiting symptoms characteristic of root disease

Table 2.2 Probability of a greater *F*-statistic for seedling health parameters, root collar diameter (RCD), stem biomass, fine root biomass, the transformed variables, square root of stem biomass and fine root biomass.

Source	df	RCD (mm)	Stem Biomass (g)	Square-root Stem Biomass	Fine Root Biomass (g)	Square-root Fine Root Biomass
Replication	1	0.0001	0.2034	0.0679	0.0001	0.0001
Treatment	4	0.0029	0.1039	0.0526	0.0052	0.0011
Tree Species	2	0.0001	0.0001	0.0001	0.0001	0.0001
Treatment x Tree Species	8	0.0001	0.0079	0.0025	0.2497	0.1801
Error	*1070*					

Table 2.3 Root collar diameter (RCD), stem biomass, and transformed variable square-root of stem biomass for each tree x treatment combination.

Tree Species	Treatment	RCD (mm)	Stem Biomass (g)	Square-Root Stem Biomass
	Control	5.40 (1.20)d	6.91 (3.60)	2.55 (0.64)c
	G. huntii	5.31 (1.04)d	6.89 (2.93)	2.56 (0.59)c
P. taeda	*L. serpens*	5.35 (1.05)d	6.67 (2.92)	2.52 (0.55)c
	L. terebrantis	5.68 (1.18)d	6.71 (2.77)	2.53 (0.55)c
	L. procerum	5.51 (1.24)d	6.66 (2.66)	2.53 (0.51)c
	Average	5.45 (1.15)	6.77 (2.98)	2.54 (0.57)
	Control	5.40 (1.36)de	7.03 (3.68)	2.55 (0.71)c
	G. huntii	5.31 (1.46)d	7.06 (4.03)	2.54 (0.80)c
P. elliottii	*L. serpens*	4.66 (1.43)f	5.12 (3.04)	2.16 (0.68)c
	L. terebrantis	4.79 (1.18)ef	5.58 (2.84)	2.27 (0.67)c
	L. procerum	6.00 (1.54)d	7.87 (4.04)	2.72 (0.71)c
	Average	5.29 (1.47)	6.53 (3.68)	2.45 (0.74)
	Control	11.09 (2.79)b	10.85 (5.78)	3.18 (0.86)ab
	G. huntii	10.59 (3.38)c	10.23 (4.78)	3.11 (0.78)b
P. palustris	*L. serpens*	11.61 (3.34)ab	12.03 (6.26)	3.36 (0.88)a
	L. terebrantis	11.74 (2.94)a	11.19 (4.65)	3.26 (0.74)ab
	L. procerum	11.68 (3.27)a	11.96 (6.21)	3.35 (0.88)ab
	Average	11.34 (3.16)	11.26 (5.62)	3.25 (0.83)

Note**:** Means (followed by standard deviation in parentheses) within a column with the same letter are not significantly different from one another at the 0.05 level.

Table 2.4 Biomass of all lateral and fine roots as well as the square-root of the root biomass for each tree species.

Tree Species	Fine Root Biomass (g)	Square Root FR Biomass
P. taeda	1.91 (1.14)	1.32 (0.40)b
P. elliottii	1.91 (1.14)	1.19 (0.48)c
P. palustris	2.95 (2.24)	1.60 (0.62)a

Note**:** Means (followed by standard deviation in parentheses) within a column with the same letter are not significantly different from one another at the 0.05 level.

Table 2.5 Biomass of all lateral and fine roots as well as the square-root of the root biomass for each treatment.

Treatment	Fine Root Biomass (g)	Square Root FR Biomass
Control	2.33 (1.82)	1.42 (0.56)ab
G. huntii	1.97 (1.47)	1.30 (0.52)b
L. serpens	1.95 (1.60)	1.29 (0.54)b
L. terebrantis	2.17 (1.43)	1.39 (0.48)ab
L. procerum	2.42 (2.05)	1.46 (0.55)a

Note**:** Means (followed by standard deviation in parentheses) within a column with the same letter are not significantly different from one another at the 0.05 level.

Table 2.6 Probability of a greater *F*-statistic for survival, lesion presence, re-isolation, lesion length, and occlusion length following inoculation with four ophiostomatoid fungi.

Source	df[†]	Survival	df[‡]	Lesion	Re-Isolation	df[§]	Lesion Length	Occlusion Length
Replication	1	0.9861	1	0.7654	0.0345	1	0.0001	0.0001
Treatment	4	0.7177	3	0.0087	0.0920	3	0.0001	0.0001
Tree Species	2	0.0003	2	0.0001	0.0028	2	0.0001	0.0001
Treatment x Tree Species	8	0.1431	6	0.0066	0.9339	6	0.0001	0.0001
Error	14		11			853		

[†]Control seedlings were included in the analysis.

[‡]Control seedlings were omitted from the analysis.

[§]All living seedlings were included in analysis.

Table 2.7 Seedling survival, lesion occurrence, lesion length, sapwood occlusion length and pathogen re-isolation frequency after 12 weeks following inoculation.

Tree Species	Treatment	Survival (%)	Lesion (%)	Lesion Length (mm)	Occlusion Length (mm)	Re-Isolation (%)
	Control	91 (3)	NA	NA	NA	NA
	G. huntii	96 (6)	100 (0)a	21 (7.06)b	33 (9.83)a	89 (9)
P. taeda	*L. serpens*	98 (4)	100 (0)a	19 (5.45)c	28 (7.63)b	81 (2)
	L. terebrantis	99 (2)	100 (0)a	17 (5.17)de	25 (7.41)c	85 (6)
	L. procerum	99 (2)	100 (0)a	15 (4.32)e	22 (6.29)d	91 (13)
	Average	**96 (4)a**	**100 (0)**	**18 (8.85)**	**27 (13.14)**	**86 (8)a**
	Control	80 (3)	NA	NA	NA	NA
	G. huntii	84 (16)	100 (0)a	24 (11.34)a	32 (13.18)a	83 (7)
P. elliottii	*L. serpens*	63 (1)	100 (0)a	18 (9.50)cd	25 (13.29)c	79 (15)
	L. terebrantis	81 (21)	98 (0.2)a	13 (6.37)f	19 (9.65)d	88 (14)
	L. procerum	67 (4)	100 (0)a	13 (7.28)f	17 (9.50)de	96 (6)
	Average	**75 (12)b**	**99 (1)**	17 (10.17)	23 (13.48)	86 (11)a
	Control	82 (5)	NA	NA	NA	NA
	G. huntii	71 (12)	98 (1)a	10 (6.06)g	16 (9.46)ef	62 (6)
P. palustris	*L. serpens*	86 (6)	98 (3)a	10 (5.22)g	14 (7.20)f	83 (16)
	L. terebrantis	87 (1)	85 (7)b	7 (5.41)h	11 (7.31)g	70 (14)
	L. procerum	92 (1)	80 (7)b	6 (4.69)i	10 (7.11)g	79 (12)
	Average	**84 (9)b**	**90 (9)**	8 (5.59)	13 (8.25)	68 (12)b

Note: Means, followed by standard deviation in parentheses within a column with the same letter are not significantly different from one another at the 0.05 level.

Table 2.8 Probability of greater F-statistic for predawn and midday water potential measurements.

Source	df	Predawn	Midday
Measurement Day	7	0.0001	0.0001
Treatment	4	0.6830	0.9342
Tree Species	2	0.0001	0.0001
Treatment x Tree	8	0.4335	0.9977
Error	*128*		

Table 2.9 Mean predawn and midday water potential for each tree species.

Tree Species	Needle $\Psi_{predawn}$ (Mpa)	Needle Ψ_{midday} (Mpa)
P. taeda	-0.52a	-1.41a
P. elliottii	-0.49a	-1.26a
P. palustris	-0.39b	-0.94b

Note: Means within a column with the same letter are not different from one another at alpha = 0.05 based on the Tukey-Kramer multiple comparison test.

Fig. 2.1 Loblolly pine seedling wound inoculated with *L. terebrantis* in the lower stem.

Fig. 2.2 Darkened lesion following inoculation with *L. serpens*.

Fig 2.3 Callus tissue formed surrounding the point of inoculation with *L. procerum* (left) and *L. terebrantis* (right).