Comparative behavior of root pathogens in stems and roots of southeastern *Pinus* species

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Abstract

With the increasing potential for accidental pest introductions and climate change, root disease is expected to become more severe in the future, increasing the need for accurate and efficient inoculation tests. For many root pathogens, these tests have been conducted in stems instead of roots. Since the relationship between root and stem inoculations are rarely compared, the applicability of results from stem inoculations is unclear. We examined root and stem damage following controlled inoculations with five root pathogens, including *Grosmannia*

huntii, *G. alacris*, *Leptographium procerum*, *L. terebrantis*, and *Heterobasidion irregulare*, and two *Pinus* hosts in the southeastern United States. Each test species was also grown on water agar amended with root and stem tissue to assess growth potential on each carbohydrate source. The relationship between root and stem damage following inoculation was similar among most combinations of pathogens in all but one experiment. Most fungal species caused greater damage in roots compared to stems in both pine hosts. Results from growth of the fungi on amended agar tended to go against the findings from tree inoculation tests, with greater growth in stem tissue for many species. This examination suggests stem inoculations would be a useful surrogate when comparing the pathogenicities of multiple pathogens. However, stem inoculations are limited to relative pathogenicity assessments, since damage in roots and stems is not comparable for most species. Finally, results from *G. huntii* inoculations illustrate important behavioral differences within different hosts.

Keywords: pathogenicity, root disease, annosus, blue-stain, loblolly, pine

1. Introduction

Root disease represents a significant disturbance in natural and plantation forests around the world. In natural stands, root disease can directly alter forest composition, structure (Rizzo and Slaughter 2001), and function (Hansen and Goheen 2000). In forest tree plantations, root disease can cause significant losses both through growth reduction and tree mortality (Bendz-Hellgren and Stenlid 1997; Froelich et al. 1977). For example, losses for annosus root disease in white fir (*Abies concolor* [Gordon] Lindley ex Hildebrand) were reported as being 69% in heavily managed stands (Goheen and Goheen 1989). Root disease may also interact with other disturbance factors, including fire, insects, other diseases, and climate change to contribute to additional tree mortality (Alexander et al. 1980; Bergot et al. 2004; Parker et al. 2006). Increases

in global trade and travel in recent decades have resulted in the exponential increase in introductions of exotic disease pathogens to forest ecosystems (Liebhold et al. 2012; Roy et al. 2014). Introduced root pathogens can cause catastrophic damage to native forests (Podger 1972). With the increasing threat from the introduction and movement of exotic root pathogens (Pimentel et al. 2000, Wingfield et al. 2001) as well as interactions between disease and climate change (Sturrock et al. 2011), root diseases are likely to pose a greater threat to both native and plantation forest stands in future.

To understand and predict the potential impact of root pathogens, accurate methods for examining the interaction between pathogen, host, and environment are necessary. Inoculation tests have historically provided an efficient experimental approach to study these relationships (Wright 1933; Hansen et al. 2005). Root disease in forest tree species have been examined through inoculation tests of both seedlings (Robin and Desprez-Loustau 1998) and mature trees (Camy et al. 2003). Due to the difficulties associated with mature tree inoculations of roots, stem inoculations are often used as a surrogate (Shain 1967; Bodles et al. 2007; Nagy et al. 2006). There are, however, potentially important anatomical (Aloni 1987), physiological (Sung et al. 1996), and environmental differences between root and stem tissue (Bert and Danjon 2006) that should be considered. Fungal growth, survival, and host response to invading fungi may also be different between root and stem tissue for certain species (Bunny et al. 1995; Parke and Lewis 2007).

A variety of root diseases impact the extensive pine-dominated forests of the southeastern United States (Tainter and Baker 1996), collectively representing a significant disturbance agent. Annosus root disease, caused by the endemic pathogen *Heterobasidion irregulare* (Fr.) Garbelotto & Otrosina (previously *H. annosum* (Fr.) Bref. [Otrosina and Garbelotto 2010]), and

Littleleaf disease, caused by the exotic pathogen *Phytophthora cinnamomi* Rands, have historically affected natural forest and plantation trees across the gulf coastal plain and piedmont physiographic regions, respectively (Bogges and Newman 1947; Stambaugh 1989). More recently, root disease associated with a combination of native and exotic ophiostomatoid fungi has been considered a contributor to the pine decline syndrome (Eckhardt et al. 2010). Common symptoms described for these disorders include root deterioration leading to thinning foliage, needle shortening, and tree growth reductions (Campbell and Copeland 1954; Asiegbu et al. 2005; Eckhardt et al. 2007).

A variety of inoculation experiments have been undertaken with root pathogens in the southeastern United States in order to examine their relationships with various hosts and environmental conditions (Kuhlman 1969; Hendrix and Campbell 1968; Eckhardt et al. 2004). Most inoculation experiments have been conducted using 9-12 month-old tree seedlings (Koenigs 1970; Hendrix and Campbell 1968; Matusick and Eckhardt 2010). Tree seedling inoculations have proven useful for investigating variability in pathogenicity among fungal species (Eckhardt et al. 2004), isolates (Kuhlman 1970), host tree species (Matusick and Eckhardt 2010), tree families (Singh et al. 2014), and interactions between disease and environmental conditions (Koenigs 1970; Matusick et al. 2008). Inoculation of mature trees, under natural conditions, is likely more useful for investigating disease development and progression since mature trees can react differently than seedlings to infection (Johansson et al. 2004). A variety of mature tree inoculations have been conducted with southeastern root pathogens (Hendrix and Kuhlman 1964; Eckhardt et al. 2004; Matusick et al. 2010). Though most inoculations of mature trees in the southeastern United States have been conducted on their roots (Hendrix and Kuhlman 1964; Matusick et al. 2010; Matusick et al. 2012), inoculation tests

using the same pathogens on hosts located outside the southeastern United States are regularly undertaken on above-ground tissue (Bodles et al. 2007; Johansson et al. 2004; Krekling et al. 2004). Despite this, the location of inoculation (root versus stem) as a source of variation has not been adequately examined for root pathogens, particularly within the southeastern United States. A series of field inoculation studies and laboratory experiments were conducted in order to answer three primary research questions: (1) Can stem inoculations be used as a surrogate for root inoculations when comparing the pathogenicities of multiple root pathogens? (2) Do fungal root pathogens cause greater tissue damage in roots compared to stems following inoculation? (3) Can fungal growth in agar amended with root and stem tissue explain results from field inoculations of roots and stems?

2. Materials and Methods

2.1. Field inoculation studies

Inoculations were conducted in two even-age, plantation-grown *Pinus palustris* Mill. and *P. taeda* L. stands at Fort Benning Military Reservation, Georgia. In 2008, trees from one *P. palustris* stand and one adjacent *P. taeda* stand (14 years old) located in the S1 training area (Lat 32.370141, Long -84.852426) were used. In 2009 the experiment was repeated in adjacent *P. palustris* and *P. taeda* pine (16 years old) stands in training area T5 (Lat 32.389207, Long -84.788923). Both experiments were conducted in the spring season (April-May). All forest stands had experienced frequent (2 or 3 years), low intensity, dormant season controlled burns since planting, including approximately 18 months prior to each experiment. All trees chosen for inoculations were free of noticeable disease and insect damage, had healthy crown foliage, and had recently flushed during the growing season.

A total of six inoculation treatments were used, including five fungal species and a wound control. The five fungal species included *Heterobasidion irregulare* and four ophiostomatoid fungi associated with root disease in trees experiencing pine decline, including *Grosmannia huntii* (R.C. Rob. Jeffr.) Zipfel, Z.W. de Beer & M.J. Wingf. (formerly *Ophiostoma huntii* [Zipfel et al. 2006])(*L. huntii* M.J. Wingfield), *L. procerum* (Kendrick) M.J. Wingfield, *Grosmannia alacris* T.A.Duong, Z.W.de Beer & M.J. Wingf (Duong et al. 2012) (formerly *L. serpens* [Goidanich] Siemaszko), and *L. terebrantis* S.J. Barras & T.J. Perry. A single isolate of each species was used for inoculation tests, originally collected from southeastern pine roots using sampling methods as described in Eckhardt et al. (2007). Isolates were identified both morphologically and phylogenetically with the assistance of Prof. M.J. Wingfield at the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa. Identical isolates have been used in several previously reported studies (Eckhardt et al. 2009; Matusick and Eckhardt 2010; Matusick et al. 2010) and are stored in the Forest Health Dynamics Laboratory culture collection at Auburn University. All fungal isolates were grown for seven days on pine tissue amended water agar after transfer of a single hyphal tip, and were actively growing prior to their use as inoculum. Since a limited number of trees were available for use, multiple fungal species were included on the same tree, similar to previous studies (Matusick et al. 2010; Matusick et al. 2012). In order to distribute fungal isolates evenly, all fungal species were paired with one another and randomly designated as species A and B for analysis. This resulted in ten total fungal pairings. Each fungal pairing was randomly assigned to five trees within each stand of *P. taeda* and *P. palustris* for both 2008 and 2009. On each tree, inoculations were conducted on both the stem and primary lateral roots with the assigned treatments. In addition, a wound control was included on one lateral root and on the stem of each treated tree. For stem

inoculations, two fungal treatment inoculations and one wound control were distributed equidistant from each other with randomly assigned aspects and treated at breast height (1.37 m) (Fig. 1). Three primary lateral roots were then excavated one meter from the base of the tree and each treatment (two fungal species and one wound control) was randomly placed on each root approximately 30 cm from the base of the tree. All inoculations were conducted using methods described by Wright (1933) for inoculating large trees. A 13 mm diameter leather punch was used to create a wound, exposing the cambium. A 10 mm diameter plug of actively growing mycelium was placed against the surface of the cambium and the excised plant tissue was placed over the mycelium. For controls, the tissue was wounded in an identical fashion and nothing was placed against the cambial surface. All treatment points were covered with duct tape and the excavated roots buried.

After eight weeks, all inoculations were examined for damage. All treated roots were re-excavated, excised from the tree, and removed from the ground for dissection and measurements. Bark tissue was removed from the area surrounding each treatment point, exposing the damaged tissue. A clear transparent sheet was used to trace the outline of the lesion that resulted. An identical procedure was used to dissect stem inoculations. The surface area of all traced lesions was measured using a Lasico® planimeter as described in Klepzig and Walkinshaw (2003). Small pieces of tissue were removed from the areas surrounding each inoculation point for re-isolation. Tissue samples were later surface sterilized and placed on 2% malt extract agar for fungal identification.

2.2. Laboratory fungal growth experiment

Stem and primary root tissue were obtained from mature (> 30 cm DBH) and sapling-sized (< 5 cm DBH) *P. taeda* and *P. palustris* trees. All tissue was cut into sections approximately 3cm x 1cm x 0.5cm (LxWxD), sterilized twice using an autoclave, and amended to water agar Petri plates. Each fungal species was transferred using a single hyphal tip to twelve replicate plates of each tree species x tissue location combination. Growth plates were incubated in the dark at 23°C. Total colony growth was traced onto transparent sheets at 4, 6, and 8 days following fungal transfer. Total colony growth was measured and calculated using a Lasico® planimeter.

2.3. Statistical Analysis

2.3.1. *Can stem inoculations be used as a surrogate for root inoculations when considering the pathogenicity of root pathogens?*

The damage (lesion area) resulting from control inoculations was subtracted from each fungal treatment inoculation in roots and stems for each tree, resulting in the damage from fungal inoculation alone. Next, the difference in lesion area was determined for each fungal treatment pair in stems and roots separately, resulting in one value for root and stem inoculations on each tree. Analyses were conducted separately for *P. taeda* and *P. palustris*. A Lavene's homogeneity of variance test was used to determine whether the data from the two experiment years could be combined using PROC GLM in SAS statistical software (SAS institute, 9.1 ed., Cary, NC). To test for the surrogacy of root and stem inoculation techniques for the fungal pairings, data were analyzed using a split-plot ANOVA in PROC MIXED in SAS, including treatment pair, inoculation location (root/stem) and their interaction in the model.

2.3.2. *Do fungal root pathogens cause greater damage in roots or stems following inoculation?*

Measurements from control inoculations were subtracted from each fungal treatment inoculation for both roots and stems on each tree. Root and stem lesion measurements (lesion area) were compared for each fungal species x tree species separately using a paired t-test in SAS.

2.3.3. *Does fungal growth in agar amended with root and stem tissue explain results from field inoculations of roots and stems?*

Results from the laboratory growth experiment were examined for each fungal species separately using repeated measures ANOVA in the GLM procedure in SAS, including tree species, tissue location, and their interaction as factors.

3. Results

Darkened, clearly defined, resin-filled lesions were consistently observed surrounding inoculations with fungi in both roots and stems of *Pinus* species (Fig. 2). In the most severely damaged roots, large fissures were observed penetrating deep into the sapwood. Severe stem damage was represented by long darkly stained tissue overlaying pockets of resin in the sapwood. The control treatment caused lightly stained tissue surrounding treatment points in both roots and stems, which resulted in relatively small lesion areas in roots and stems of both pine species (Table 1). Fungal species were readily re-isolated from areas within the darkly stained tissue, including near the lesion margin. Some samples were damaged during the re-excavation procedure and were not used in analyses. From the original 40 fungal treatment points, the

following resulted for *P. taeda* and *P. palustris* (*P.taeda/P.palustris*): *H. irregulare* (40/36), *G. huntii* (38/37), *L. procerum* (36/39), *G. alacris* (36/38), *L. terebrantis* (38/38).

3.1. Can stem inoculations be used as a surrogate for root inoculations when considering the pathogenicity of root pathogens?

From Lavene's test it was determined that data from experiment years could be combined for *P. palustris* ($F_1=2.79$, $P=0.0968$) but not *P. taeda* ($F_1=5.23$, $P=0.0234$). Therefore future analyses were conducted separately for *P. taeda* for both experiment years. For *P. taeda* inoculations in 2008, a significant interaction between treatment pair and inoculation location was detected ($F_9=3.50$, $P=0.0032$). However, using data from 2009 significant main effects were found for treatment pair ($F_9=7.70$, $P<0.0001$) and inoculation location ($F_1=7.74$, $P=0.0085$) separately. For *P. palustris* inoculations a significant interaction was detected between treatment pair and inoculation location ($F_9=4.60$, $P<0.0001$).

3.2. Do fungal root pathogens cause greater damage in roots or stems following inoculation?

Certain fungal pathogens caused greater damage in roots, while other species caused greater damage in stems following inoculation (Table 2). *Heterobasidion irregulare* and *G. alacris* caused significantly greater lesion areas in roots compared to stems in both pine species. *Leptographium procerum* also created larger lesions in roots, though not significantly greater in *P. palustris* (3.59 cm^2 , $T=1.91$, $P=0.0631$). In contrast, *L. terebrantis* caused larger lesions in stems compared to roots, though not significantly so in either tree species. *Grosmannia huntii* caused significantly larger lesions in roots of *P. taeda*, while causing larger lesions in stems of *P. palustris*.

3.3. Does fungal growth in agar amended with root and stem tissue in vitro explain results from field inoculations of roots and stems?

Results from the repeated measures ANOVA found significant interactions between time of measurement (4, 6, 8 days) and tissue type (root versus stem) for *H. irregulare* and *L. procerum* in both pine species, and *L. terebrantis* using *P. taeda* tissue only (Table 3). Additionally, growth of *G. huntii* and *L. terebrantis* on *P. palustris* stem versus root tissue was significantly different (Table 4). There was no significant difference in the growth of *G. alacris* on root versus stem tissue in either pine species.

Significantly greater fungal growth was observed in stem tissue compared to root tissue when considering *H. irregulare* and *L. procerum* in both *P. taeda* and *P. palustris* (Fig. 3). Growth of *G. huntii* in *P. palustris* root tissue was significantly greater than in stem tissue over all measurement periods. Growth was, however, greater in stem tissue in *P. taeda*, though not significant. *L. terebrantis* showed greater growth in root tissue for both pine species.

4. Discussion

These experiments generally show that stem inoculations can serve as useful surrogates for root inoculations when comparing the relative pathogenicities among multiple fungal root pathogens. The one exception involved inoculations of *P. taeda* in 2008, which showed significant divergence in the relationship between the root pathogens in stems and roots. It is unknown exactly why this was the case; however, the relationships between root and stem lesions were inconsistent with regards to *G. huntii* compared to the remaining fungal species. This was likely driven by the consistently large root lesions produced in *P. taeda* in 2008, which

may help to explain the different findings in this experiment. These results suggest that if independent root and stem inoculations were undertaken involving *G. huntii*, they could result in different findings with respect to the relative pathogenicity of the species. Nonetheless, for the remaining fungal species the relationship between root and stem lesions were consistent, confirming that stem inoculations could serve as useful surrogates for root inoculations with these species. Although several inoculation studies involving forest root pathogens have included both root and stem inoculations of mature trees (Davison et al. 1994; Otrosina et al. 1999; Shearer et al. 1987), few directly compare the two methods. Davison et al. (1994) found no statistical difference between lesion lengths in stems and roots following *P. cinnamomi* inoculation. Shearer et al. (1987) tested a variety of *Phytophthora* species on excised root and intact stems. Though no formal comparison was made between the two methods, both resulted in similar relative pathogenicities among the tested species (Shearer et al. 1987). In the southeastern United States, Otrosina et al. (1999) inoculated both stems and roots of *P. palustris* with a variety of ophiostomatoid fungi, including *L. procerum* and *L. terebrantis*. However, no formal comparison was made between methods, although lesion results appeared to be similar among roots and stems for most species tested (Otrosina et al. 1999).

Although the relationship between root and stem lesions allows for useful comparisons between fungal species, important differences were observed for many fungal pathogens between root and stem lesions. This represents the only study that we are aware of that contrasts root and stem inoculation results using ophiostomatoid fungi and *H. irregulare*. Examinations of this type using other root disease pathogens have, however, found similar differences between root and stem lesions (Shearer et al. 1987). A cross-study examination of root (Matusick et al. 2012) and stem inoculations (Eckhardt et al. 2004) in *P. taeda* suggests lesions would be larger for *G.*

alacris, *L. terebrantis*, and *L. procerum* in stems. This contrasts with the findings reported in this study, which show that *G. alacris* and *L. procerum* produce larger lesions in roots. Although root and stem damage is proportional in most species, the actual differences between root and stem lesions for many fungal species suggest that stem inoculation methods would not provide accurate estimates of tissue damage in roots following inoculation.

Ecological niche could help to explain the contrasting results related to virulence of fungal pathogens in roots and stems for certain species. For example, *H. irregulare* caused larger lesions in roots compared to stems. *Heterobasidion irregulare* is considered to be a structural root pathogen, invading healthy roots either through root grafts with infected stumps (Otrosina and Cobb 1989) or by direct infection from spores (Asiegbu et al. 1993). Although *H. irregulare* can readily infect stumps above-ground, disease caused by *H. irregulare* is thought to be limited to roots (Bega 1963). Previous inoculation attempts with *H. irregulare* in stems also found relatively small lesions following inoculation (Nagy et al. 1996). Other studies have suggested that the volume and composition of resin produced in response to infection in the stem does not allow for growth of *H. irregulare* and disease development (Shain 1967). Although no effort was made to measure resin response directly, both stems and roots were observed producing copious amounts of resin in response to *H. irregulare* inoculation in this study, which has been observed previously (Hodges 1969). This collection of evidence suggests *H. irregulare* may perform better in roots due to other differences between root and stem tissue. Similarly, *G. alacris* is generally found invading root tissue of living trees (Wingfield and Marasas 1980; Heiniger et al. 2011), which supports the pattern with respect to virulence in root versus stem tissue reported in this study. Though it can be found in above-ground tissues elsewhere (Peverieri et al. 2006), *G. alacris* has only been observed invading and causing disease in root

tissues of mature trees in the southeastern United States. Similar to *H. irregulare*, *G. alacris* may likely be better adapted to the environment within roots compared with stems. The only species that did not cause larger lesions in roots of at least one pine species was *L. terebrantis*. Similar to the other ophiostomatoid fungi used, *Leptographium terebrantis* is vectored by a suite of wood-boring beetle and weevil species (Jacobs and Wingfield 2001). In contrast to the other ophiostomatoid species, *L. terebrantis* is commonly vectored by the black turpentine beetle (*Dendroctonus terebrans* Oliv.)(Coleoptera: Curculionidae)(Barras and Perry 1971), which primarily invades the stem of trees. Not surprisingly, *L. terebrantis* is commonly observed invading stem tissue in the field (Rane and Tattar 1987). Additional research is thus required to examine the potential differences between pine root and stem tissues and their relationship to defense against these root pathogens.

Patterns observed with respect to pathogen growth in agar amended with root and stem tissue largely contrasted with results obtained from previously published field inoculation studies, which found growth on agar correlated with length of the reaction zone in field-grown trees (Uzunović and Webber 1998; Lieutier et al. 2004). For example, *G. huntii* caused larger lesions in the stems of *P. palustris*, while growing best in agar amended with root tissue. Similarly, *H. irregulare* and *L. procerum* grew best in stem tissue but caused greater damage in roots of mature trees. To the best of our knowledge this is the first study to compare pathogen growth in agar with root and stem tissue as the carbohydrate source. Additional research is necessary to examine differences in both structural defense and accessibility of carbohydrates between the two tissue types. Additionally, it is important to recognize that lesions measured following inoculation represent the combination of pathogen infection and the hypersensitive response (Cook and Hain 1985; Viiri et al. 2001). Provided this fact, the contrasting results

between these two studies may suggest that the hypersensitive response may better explain the tissue damage observed in the field, as opposed to fungal growth.

The relationship between root and stem damage following inoculation following *G. huntii* inoculation strongly contrasted between the two pine hosts, which was not observed with the other species. It is not clear why these contrasting results were observed. Future work should explore possible explanations for the divergent pattern between *P. taeda* and *P. palustris* with respect to *G. huntii* virulence.

4.1. Conclusion

With the potential for increased damage from new and emerging root disease threats around the world, it is important to both accurately identify efficient methods for examining host susceptibility. This study suggests that stem inoculations provide a useful alternative to root inoculations when multiple fungal species are being compared. However results confirm that for most root pathogens, stem damage following inoculation is neither similar nor proportional to root damage, and root inoculations provide the most realistic damage assessment. Finally, results suggest that the ability of a given species to colonize and cause disease in root or stem tissue aligns with its ecological niche.

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Figure captions

Figure 1. A diagram, to illustrate the inoculation treatment pattern within each of the treated tree. Each tree received two fungal treatments (two different fungal species) and one wound control. Each treatment was performed on the stem and on one of the primary lateral root.

Figure 2. Darkened, resin-filled tissue in (a) tree stem and (b) lateral root surrounding the point of inoculation with *Grosmannia huntii* eight weeks following treatment.

Figure 3. Total fungal colony growth (cm²) on water agar amended with pine tissue 4, 6, and 8 days following transfer. Species used include *Heterobasidion irregulare* (a) and (b), *Grosmannia huntii* (c) and (d), *Leptographium procerum* (e) and (f), *G. alacris* (g) and (h), and *L. terebrantis* (i) and (j).

Asterisks denote significant differences at $P < 0.05$ between root and stem tissue from a post hoc Tukey's honest significant difference (HSD) test. Numbers in parentheses represent average daily growth rates.

Table 1. The mean lesion area ($\pm$ standard error (SE)) eight weeks following treatment of mature *Pinus taeda* and *P. palustris* roots and stems with five fungal pathogens and a wound control.

Tree Species	Inoculation Site	Treatment	Lesion Area (cm ²)
<i>Pinus taeda</i>	Root	Control	4.33 $\pm$ 0.19
		<i>Heterobasidion irregulare</i>	17.74 $\pm$ 1.70
		<i>Grosmannia huntii</i>	38.72 $\pm$ 3.65
		<i>Leptographium procerum</i>	11.35 $\pm$ 0.82
		<i>Grosmannia alacris</i>	28.96 $\pm$ 1.79
		<i>Leptographium terebrantis</i>	14.92 $\pm$ 0.90
	Stem	Control	4.02 $\pm$ 0.25
		<i>Heterobasidion irregulare</i>	10.89 $\pm$ 1.62
		<i>Grosmannia huntii</i>	18.85 $\pm$ 1.43
		<i>Leptographium procerum</i>	8.48 $\pm$ 0.63
		<i>Grosmannia alacris</i>	21.44 $\pm$ 2.60
		<i>Leptographium terebrantis</i>	17.39 $\pm$ 1.33
<i>Pinus palustris</i>	Root	Control	4.16 $\pm$ 0.14
		<i>Heterobasidion irregulare</i>	15.57 $\pm$ 1.96
		<i>Grosmannia huntii</i>	22.66 $\pm$ 1.80
		<i>Leptographium procerum</i>	13.29 $\pm$ 1.55
		<i>Grosmannia alacris</i>	24.67 $\pm$ 2.29
		<i>Leptographium terebrantis</i>	15.67 $\pm$ 1.24
	Stem	Control	3.58 $\pm$ 0.24
		<i>Heterobasidion irregulare</i>	9.70 $\pm$ 0.72
		<i>Grosmannia huntii</i>	31.36 $\pm$ 2.65
		<i>Leptographium procerum</i>	9.30 $\pm$ 1.68
		<i>Grosmannia alacris</i>	18.39 $\pm$ 2.05
		<i>Leptographium terebrantis</i>	18.11 $\pm$ 1.41

Table 2. Mean difference between lesions ($\pm$ SE) formed in roots and stems eight weeks following inoculation with five fungal treatments in *Pinus taeda* and *P. palustris* trees. Effects significant at the 5% level are highlighted in bold.

Fungal treatment		Root lesions (cm ²)	Stem lesions (cm ²)	Root-stem lesions (cm ²)	<i>T</i> -value ^a	<i>P</i> -value
<i>H. irregulare</i>	<i>P. taeda</i>	13.05 $\pm$ 1.77	6.94 $\pm$ 1.77	6.10 $\pm$ 2.30	2.65	0.0114
	<i>P. palustris</i>	11.53 $\pm$ 1.83	6.42 $\pm$ 1.83	4.29 $\pm$ 1.94	2.21	0.0332
<i>G. huntii</i>	<i>P. taeda</i>	34.70 $\pm$ 1.86	14.93 $\pm$ 1.86	19.78 $\pm$ 4.02	4.91	<0.0001
	<i>P. palustris</i>	18.47 $\pm$ 1.81	27.93 $\pm$ 1.81	-9.60 $\pm$ 3.31	-2.91	0.0061
<i>L. procerum</i>	<i>P. taeda</i>	7.12 $\pm$ 1.89	4.56 $\pm$ 1.89	2.61 $\pm$ 0.96	2.70	0.0104
	<i>P. palustris</i>	9.10 $\pm$ 1.76	5.25 $\pm$ 1.76	3.59 $\pm$ 1.87	1.91	0.0631
<i>G. alacris</i>	<i>P. taeda</i>	24.68 $\pm$ 1.86	17.70 $\pm$ 1.86	6.98 $\pm$ 3.20	2.18	0.0359
	<i>P. palustris</i>	20.65 $\pm$ 1.78	14.40 $\pm$ 1.78	5.88 $\pm$ 2.47	2.38	0.0223
<i>L. terebrantis</i>	<i>P. taeda</i>	10.50 $\pm$ 1.86	12.79 $\pm$ 1.86	-2.39 $\pm$ 1.69	-1.42	0.1642
	<i>P. palustris</i>	11.34 $\pm$ 1.78	14.84 $\pm$ 1.78	-3.50 $\pm$ 1.84	-1.90	0.0648

^a. *T* and *P*-values are the result of paired t-tests performed using ESTIMATE statements in the GLM procedure in SAS

Table 3. Repeated measure ANOVA results following a fungal species growth assay on *Pinus taeda* root and stem tissue (tissue type (TT)).

<i>Pinus taeda</i>	<i>H. irregulare</i>	<i>G. huntii</i>	<i>L. procerum</i>	<i>G. alacris</i>	<i>L. terebrantis</i>
	<i>F</i> -value	<i>F</i> -value	<i>F</i> -value	<i>F</i> -value	<i>F</i> -value
Rep	0.19	0.41	0.27	0.11	1.47
Tissue type (TT)	1.34	2.90	8.71**	0.08	5.95**
Growth	579.70***	239.54***	383.08***	177.61***	442.37***
Growth. Rep	0.18	0.86	0.54	0.30	0.60
Growth. TT	3.39*	0.38	5.37**	1.94	4.21*

* Denotes significance at $P < 0.05$, ** significance at $P < 0.01$, *** significance at $P < 0.001$

Table 4. Repeated measure ANOVA results following a fungal species growth assay on *Pinus palustris* root and stem tissue (tissue type (TT))

<i>Pinus palustris</i>	<i>H. irregulare</i>	<i>G. huntii</i>	<i>L. procerum</i>	<i>G. alacris</i>	<i>L. terebrantis</i>
	<i>F</i> -value	<i>F</i> -value	<i>F</i> -value	<i>F</i> -value	<i>F</i> -value
Rep	0.14	1.61	0.17	0.63	0.13
Tissue type (TT)	7.67**	12.51**	13.07**	1.44	7.32**
Growth	802.45***	549.55***	293.67***	605.53***	309.92***
Growth. Rep	0.78	1.51	0.53	0.50	0.38
Growth. TT	5.42**	3.26	9.46**	2.57	0.25

* Denotes significance at $P < 0.05$, ** significance at $P < 0.01$, *** significance at $P < 0.001$

Figure 1

Figure 2

Figure 3