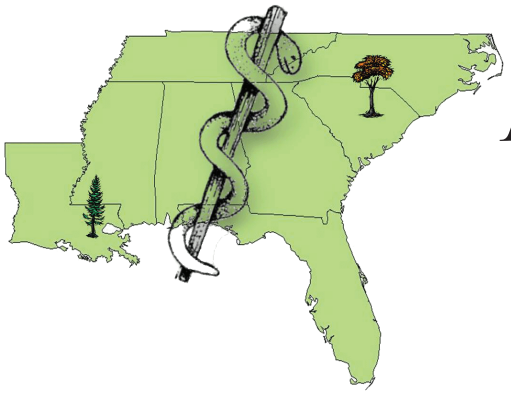

AUBURN UNIVERSITY
FOREST HEALTH COOPERATIVE

RESEARCH REPORT 17-06

INTRA-SPECIES VARIATION IN RESPONSE OF MATURE *PINUS TAEDA* FAMILIES TO ROOT-INFECTING OPHIOSTOMATOID FUNGI

by
Pratima Devkota and Lori G. Eckhardt

ABSTRACT
Seedling screening studies have shown intra-species variation in susceptibility of Pinus taeda (loblolly pine) to Leptographium terebrantis and Grosmannia huntii, the causal agents of Pinus species root-infection. Roots of mature P. taeda families determined as susceptible and tolerant to L. terebrantis and G. huntii by previous seedling screening trials were artificially inoculated with the same fungal isolates. Dark necrotic lesion and the vascular occlusion were recorded 8 weeks later. Families previously considered as susceptible had longer lesions and occlusions when compared to the tolerant families. The variation in susceptibility/tolerance pattern remained similar as exhibited by families at seedling trial. The studies indicate that intra-species variation in relative susceptibility of P. taeda to L. terebrantis and G. huntii remain the same regardless of the tree age.

INTRODUCTION
Pinus taeda L., an important timber species, is commercially planted on approximately 11.7 million hectares of land in the southern U.S. (Baker and Langdon, 1990; Rauscher, 2004). This species, when fertilized, can produce high per hectare wood volume yields (Fox et al., 2004) providing an all-purpose forest product such as furniture, pulpwood, composite boards, crates, boxes and pallets (Schultz, 1997). In addition, *P. taeda* stands provide habitat for wildlife including endangered species such as red-cockaded woodpecker (Jones and Hunt, 1996), and also place for wildlife watching and hunting (Poudel et al., 2016). This pine species directly or indirectly contributes $30 billion to the economy of the southern U.S. (Schultz, 1999).

A factor impacting the growth and optimal productivity of this important pine species is root-infecting pathogenic fungi, among which some are associated with Pine Decline (PD) (Otrosina et al., 1999; Eckhardt et al., 2004a). Pine Decline, a decline disease syndrome, was first observed by Brown and Mc Dowell (1968) on the Talladega National Forest in Oakmulgee Ranger District located in central Alabama, U.S. Brown and Mc Dowell (1968) reported 40-to-50-year-old *P. taeda* stands with symptoms of decline that included thinning crowns, reduced radial growth, and root deteriorations. This decline syndrome has subsequently been reported across the southeastern U.S. (Hess et al., 2002; Eckhardt et al., 2007).

The decline-disease spiral, the widely accepted model of forest decline, given by Manion (1981) involves interactions of 3 factors as follows: (i) factors bringing trees under constant stress (predisposing factors), (ii) short-term factors increasing the severity of stress (inciting factors), and (iii) factors a playing role at the end (contributing factors). In the context of PD, tree genetics and

increased slopes are the predisposing, drought and ozone are the inciting, and root-feeding bark beetles and their associated ophiostomatoid fungi are the contributing factors (Eckhardt et al., 2004a; Eckhardt et al., 2004b; Eckhardt et al., 2007; Eckhardt and Menard, 2008). Root-feeding bark beetles such as *Hylobius pales* Herbst., *Hylastes* spp. and *Pachylobius picivorus* (Germar) act as vectors in introducing their ophiostomatoid fungal associates, namely *Leptographium terebrantis* S.J. Barras and T.J. Perry, *Grosmannia huntii* R.C. Rob. Jeffr, and *Leptographium procerum* (W.B. Kendr.) M.J. Wingf., into roots of *P. taeda* trees (Eckhardt et al., 2004 a; Eckhardt et al., 2004b). *Leptographium terebrantis* and *G. huntii* were found to be more virulent to southern *Pinus* species (Matusick et al., 2010; Singh et al., 2014) thus warranting further need to study these fungi. Also, the inter-species and intra-species susceptibility of the host (a major predisposing factor) to fungi contributing to PD are still poorly known.

Ophiostomatoid fungi cause lesions in the phloem and occlusion in the xylem of artificially inoculated seedling stems (Eckhardt et al., 2004a), stems (Matusick et al., 2016), and roots (Matusick et al., 2010) of mature *P. taeda* trees. Furthermore, these fungi use sugars, defense compounds and sugars leaking from degraded cell walls to surive and proliferate inside xylem conduits (Hammerbacher et al., 2013). Concomitantly, trees synthesize defensive carbon compounds and form tyloses and structures that can compartmentalize fungal spread and infection (Yadeta and Thoma, 2013). Fungal spread and tyloses formation both disturb plant water transport (Joseph et al., 1988) resulting in tree decline and death. Moreover, investment by the tree in defense reaction occurs at the expense of radial growth (Krokene et al., 2008).

Artificial inoculation of *Grosmannia* and *Leptographium* species into roots of *P. taeda*, *P. palustris* Mill. (longleaf pine) and *P. elliotti* Englem. (slash pine) by Matusick et al. (2010) showed inter-species variation in susceptibility/tolerance. *Pinus taeda* was the most susceptible among 3 *Pinus* species. Furthermore, Singh et al. (2014) conducted a seedling screening study to examine intra-species variability of *P. taeda* tolerance to *G. huntii* and *L. terebrantis*. Results indicate that *P. taeda* families have varying levels of susceptibility/tolerance to those fungi. However, given that these fungi affect mature trees in the ecological scenario, the reliability of results obtained from families at a seedling stage is yet to be answered. To address this important question, we further sought to determine the intra-species variation in tolerance/susceptibility of mature *P. taeda* trees to *L. terebrantis* and *G. huntii* based on the seedling studies performed by Singh et al. (2014). We hypothesize that intra-species variation in tolerance/susceptibility of *P. taeda* to ophiostomatoid fungi is an inherent character of a family regardless of the tree's age.

MATERIALS AND METHOD

Experimental design

Pinus taeda stands from 4 families in Alabama and Georgia (~ 31°53'N and 85°8'W, 81.16 m above the sea level) were chosen. Among 4 families, T1 and T2 represent the families relatively tolerant to ophiostomatoid fungi based on seedling screening trials conducted by Singh et al. (2014). Conversely, S1 and S2, represent susceptible families. The study was conducted twice; once in summer 2015 (June 15 - August 15) and again in spring 2016 (March 15 - May 15). A total of 25 healthy mature *P. taeda* trees, 17-year-old (for 2015) and 18-year-old (for 2016) with no

visible signs or symptoms of aboveground disease were selected per family. Selected trees had a mean diameter at breast height and height of 19.5 cm (± 2.8 cm), and 13 m (± 2 m) respectively.

Inoculation experiment

Roots were artificially inoculated with fungal cultures consisting of single spore isolates of *L. terebrantis* (ATCC accession no. MYA-3316) and *G. huntii* (ATCC accession no. MYA-3311) maintained at 4 ºC in Malt Extract Agar (MEA) in the Forest Health Dynamics Laboratory at Auburn University. These fungal isolates were cultured on 2% MEA, two weeks prior to root inoculations. The *L. terebrantis* and *G. huntii* isolates used in the study were respectively isolated from the roots of *P. taeda* from the Talladega National Forest, Oakmulgee Ranger District, AL, USA and of *P. palustris* from the Fort Benning Military Reservation, GA, USA exhibiting symptoms of decline such as localized tissue damage and defoliating crowns as described by Eckhardt et al. (2007). Several previous artificial stem and root inoculation studies have used these isolates (Matusick et al., 2010; Singh et al., 2014; Chieppa et al., 2017).

In the field, two primary lateral roots were excavated with hand tools that had been sterilized using 70% ethanol from each *P. taeda* tree without damaging roots (Figure 1.1). On each excavated root, two wounds to the cambium layer and 30 cm horizontally apart from each other, were created by hitting a rubber mallet on a 13-mm diameter sterile steel arch punch. The root inoculation included removing the bark plug and placing the 10-mm agar plug (fungus-side-down) with actively growing fungi in the wound (Figure 1.2). There were 2 fungal treatments: *L. terebrantis, G. huntii* and 2 control treatments: wound with sterile media and wound without media. One of each fungal treatment and a control treatment were randomly paired together in each of the roots per tree. Following inoculation, the bark was replaced, and the wound sealed with duct tape to minimize further contamination. The inoculation points of the roots were marked with labeled pin flags and covered with soil.

Laboratory measurements

Eight weeks post-inoculation, the inoculated roots were re-excavated and removed from the tree (Figure 1.3). The exposed ends of the roots were painted with Drylok Latex Masonry Waterproofer (Scranton, PA, USA) and were transported to the lab at Auburn University for further processing (Figure 1.4). To observe the necrotized and occluded tissues, bark around the inoculation area was removed and painted with a solution of FastGreen stain (FastGreen FCF; Sigma Chemical Co.) (0.25 g/L of water). The necrotic tissue area on each inoculation site was traced on a clear transparent sheet, and lesion area was determined by using a Lasico Planimeter (Lasico®, Los Angeles, CA) as described by Matusick et al. (2012). The amount of unstained tissue around the fungal inoculation point determined the occlusion length. The root samples were cut transversely at the point of the inoculation and the discolored sapwood was measured as occlusion depth. Small pieces of stem tissue from the distal and proximal portions of the inoculation site were plated on MEA amended with cycloheximide and streptomycin to confirm fungal infection by re-isolation of the inoculated fungi.

Statistical analysis

Data were analyzed using multivariate analysis of variance (MANOVA) in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) using the PROC GLM statement. Family, fungal treatments and study period were kept as fixed effects. Possible interactions also were tested in the model. Root-diameter was

used as a covariate. The data met assumptions such as the normal distribution of residuals and homogeneity of variance, and did not warrant further transformation. Lesion length and area were used as the strongest response variables (Matusick et al., 2010; Singh et al., 2014). As the response variables (lesion and occlusion) differed by study period (replication), data was analyzed separately for each study period.

The general linear model

$$y_{ik} = \beta_0 + T_i + F_k + TF_{ik} + coV + \mathcal{E}_{ik} \quad (1)$$

was used, where y_{ik} is response variable such as lesion length, occlusion length, and depth of i^{th} fungal treatment in k^{th} family, coefficients β_0 is the intercept, T_i express the effect of fungal treatment, F_k express the effect of *P. taeda* family, TF_{ik} express the treatment and family interaction, root diameter was used as covariate (coV), and $\mathcal{E}_{ik}$ express the random error for i^{th} treatment in k^{th} family. Estimate statements were used to estimate the differences between the families. Graphs were created on STATISTICA 10 (Statsoft, Inc., Tulsa, OK, USA).

RESULTS

The inoculation of *L. terebrantis* and *G. huntii* into the roots of mature *P. taeda* led to necrosis and resin soaking around the initial point of inoculation (Figure 1.5 and Figure 1.7)). The inoculation of the wound and wound with sterile agar resulted in significantly smaller lesions than those caused by fungal inoculation (Figure 1.6). Families responded differently to the fungal inoculation at each study periods in terms of lesion area ($F_{(3,374)} = 10.48$, $p = <0.0001$), lesion length ($F_{(3,374)} = 7.69$, $p = <0.0001$) and occlusion length ($F_{(3,374)} = 3.68$, $p = 0.01$) (Figure 1.8). Hence, the data from summer and spring root inoculation studies were analyzed separately. The inoculation of the fungi into roots resulted in more tissue necrosis (in terms of lesion area) in the spring 2016 inoculation compared to the summer 2015 inoculation (Figure 1.9).

Summer 2015 inoculation

Families differed in their overall response to the fungal inoculation during summer 2015 ($F_{(21, 543)} = 3.01$, $p = <0.0001$) (Table 1.1). However, within a single family, the pathogenicity of the two fungi were not different ($F_{(21, 543)} = 1.07$, $p = 0.38$). *Leptographium terebrantis* and *G. huntii* did not differ in their virulence (in terms of occlusion length) ($F_{(7,179)} = 1.41$, $p = 0.21$) (Figure 1.10).

Four *P. taeda* families differed in their response to inoculated fungi in terms of the area ($p = <0.0001$) and length of the lesion ($p = 0.01$), and length ($p = 0.03$) and depth ($p = <0.0001$) of the vascular occlusion (Table 1.1). The lesion area did not differ significantly between two families (T1 and T2) tolerant to ophiostomatoid fungi ($p = 0.07$) and between two families (S1 and S2) susceptible to ophiostomatoid fungi ($p = 0.30$) (Table 1.2 and 1.3). The lesion area observed in tolerant family T2 was substantially smaller than susceptible families S1 and S2 (Table 1.2 and 1.3). Families showed a similar trend for lesion length. Susceptible family (S1) had a significantly longer occlusion length compared to tolerant families (T1 and T2) (Table 1.2 and 1.3). However, the depth and width of occlusion at the cross section underneath the fungal inoculation point were significantly shorter in susceptible families S1 and S2 compared to tolerant family T1 (Table 1.3).

The success of re-isolation of *G. huntii* from the inoculated root was 92%, 80%, 92%, and 92% for family T1, T2, S1, and S2 respectively. Similarly, for *L. terebrantis* the re-isolation success

was 100%, 96%, 88%, and 100% for family T1, T2, S1, and S2 respectively. The higher success of re-isolation of the fungi inoculated in root proved the success of fungal inoculation and root-infection.

Spring 2016 inoculation

Pinus taeda families responded differently to the fungal inoculation in spring 2016 ($F_{(21, 552)} = 4.63$, $p = <0.0001$). However, family and fungal treatment interaction were significant ($F_{(21,552)} = 2.27$, $p = <0.0001$), suggesting an overall variation in the pathogenicity of *L. terebrantis* and *G. huntii* within each family. The families responded differently to *G. huntii* and *L. terebrantis* in terms of lesion area ($p = 0.03$) and occlusion length ($p = 0.01$) (Table 1.1). *Leptographium terebrantis* caused significantly longer occlusion length than *G. huntii* in all of the families (Figure 1.10).

Area of the lesions caused by both fungi was significantly smaller in roots of tolerant families (T1 and T2) compared to the susceptible families (S1 and S2) (Table 1.4 and 1.5). The length of occlusion caused by *G. huntii* was significantly longer in the susceptible family S2 compared to all other families. However, occlusion length caused by *L. terebrantis* did not differ in between the four *P. taeda* families (Table 1.4 and 1.5). Both fungi caused lesions with a larger area and longer and wider length in susceptible family S2 followed by susceptible S1 (Table 1.5).

The success of re-isolation of *L. terebrantis* from inoculated roots was 100%, 100%, 92%, and 100% from family T1, T2, S1, and S2 respectively. Similarly, the re-isolation success of *G. huntii* was 84%, 76%, 64%, and 88%, from family T1, T2, S1, and S2 respectively. Consistent re-isolation of the fungi previously inoculated in root proved the success of fungal inoculation and infection.

DISCUSSION

This is the first study to show within species variation in susceptibility of mature *P. taeda* to ophiostomatoid fungi. Families S1 and S2 were more susceptible to root-infection caused by ophiostomatoid compared to family T1 and T2 (Figure 1.8) confirming the result of Singh et al. (2014) that found four *P. taeda* families used in the present study plus others have different levels of tolerance to root-infecting ophiostomatoid fungi. Intraspecific variation in susceptibility of mature *Pinus* species to ophiostomatoid fungi have been observed in some other conifer hosts (Rice et al., 2007a; Rice et al., 2007b). Several other researchers have reported considerable variation in susceptibility to pitch canker within *P. taeda* (Dwinell et al. 1977; Gordon et al., 1998; Schultz et al. 1990; Kelley and Williams, 1982) and *P. radiata* (Correll et al., 1991; Roux et al., 2007). The present study shows that there is potential for selecting ophiostomatoid fungi tolerant *P. taeda* families from the current southern U.S. planting stock reducing the potential losses due to PD.

The use of seedlings to screen tolerance/susceptibility of *P. taeda* families to *L. terebrantis* and *G. huntii* was questioned by Coyle et al. (2015). However, these results confirmed that the use of seedlings in screening studies is consistent and reliable. While, differences in the actual lesion size to define family susceptibility levels varies between the present study when compared to that of Singh et al. (2014) families performed similarly in both studies. Variation in susceptibility exists

within and between *Pinus* spp., and this variation must be quantified before host susceptibility/tolerance can be generalized across the entire range of this host.

In the spring, larger lesions and occlusions caused by *L. terebrantis* indicated a higher virulence level of *L. terebrantis* over *G. huntii*. Similar results were reported by Singh et al. (2014). Our results are not in agreement with the findings of Matusick et al. (2010) where they found *Grosmannia huntii* to be relatively more virulent than *L. terebrantis*. These discrepancies about fungal virulence can be attributed to the consideration of intra-species variability in *P. taeda* in our study which was not considered in the previous study. This implies that virulence of pathogens can be underestimated if the intra-species response is not integrated into the selection of disease tolerant hosts.

Lesions and occlusions were observed in all of tree roots. Similar hypersensitive response was observed in *P. taeda* following fungal inoculation in various previous studies (Matusick et al., 2008; Matusick et al., 2010; Matusick et al., 2012; Otrosina et al., 2000). The host response observed in control treated roots were significantly lower than those produced by the roots that were inoculated with fungal treatments. Similar observations were made by Singh et al. (2014) in their seedling inoculation experiment.

Lesion size determines the susceptibility of the host (Stephen and Paine, 1985; Matusick and Eckhardt, 2010; Singh et al., 2014). The introduction of ophiostomatoid fungi in *P. taeda* induces ethylene production which further regulates monoterpene production and guides the lesion formation (Popp et al., 1995). Paine et al. (1997) further concluded that higher monoterpene accumulation denotes elevated plant defense to invading insect vectors and pathogens. We, however, suggest that a smaller lesion length indicates that with a shorter response the host plant can suppress the effect of the fungal pathogen. The family that can block the fungal movement with less resin response is more tolerant to root-infecting fungi. The higher carbon investment of the tree in defense can result in the reduced radial growth (Krokene et al., 2008), alteration in conductive tissue (Joseph et al., 1998), xylem disruption and immediate mortality (Tyree and Zimmermann, 2002). We, thus suggest that the tolerant families T1 and T2 inhibit the fungi at the expense of less carbon and are thus less prone to infection and further reduction in productivity by ophiostomatoid fungi.

A number of the secondary metabolites produced by the host highly influence the ability of root pathogens to spread in the host (Eckhardt et al., 2009). Trees with fewer resin ducts are more susceptible to fungal infection and prone to attack by insect vectors compared to those with more ducts (Ferrenberg et al., 2014). It is, however, unclear whether variarion in either the resin constituents or the resin duct length and area among the families results in inhibition of the fungal growth. The amount of production, the rate of flow and the concentration of chemical content are heritable traits that guide the resin defense in pine trees (Chhatre et al., 2013; Westbrook et al., 2013). The inherent factors that direct the susceptibility and tolerance of *P. taeda* families to ophiostomatoid fungi are still unknown.

A relatively severe hypersensitive response was observed in spring inoculation compared to summer inoculation. Several studies suggest the timing of fungal inoculation influence the response of trees (Paine, 1984; Stephen and Paine, 1985; Matusick et al., 2010) and the results

greatly support our findings. Resin production is highest in the root cells formed immediately following the meristem differentiation and differ throughout the year (Berryman, 1972). Tree defense is highest in May and continues to decrease until December (Blanche et al., 1992). Thus, lesions are larger during the growing season that the dormant season (Stephen and Paine, 1985; Matusick et al., 2010). The larger lesion response requires higher carbon at the inoculation site (Guérard et al., 2007.) There is a trade-off between investment of tree in defense and decrease of the radial growth and loss of conductive tissue (Oliva et al., 2014).

Recent research has made great strides towards understanding intra-species variation in disease tolerance, but their implication in mature trees in an ecological scenario remains understudied. This paper presents the first study to show intra-species variation in disease tolerance exists in mature *P. taeda* trees. Our results suggest that the susceptibility and tolerance to *L. terebrantis* and *G. huntii* is an inherent property of families, regardless of the life stage of *P. taeda*. The family genetics, a significant role player in susceptibility and tolerance to pathogens, should be considered in future studies. It is suggested that future studies of the molecular, anatomical and chemical mechanism of defense strategies will improve our understanding of these findings.

REFERENCES

Baker, J. B., and Langdon, O. G., (1990). *Pinus taeda* L. Silvics of North America. 1, 497-512.

Berryman, A. A. (1972). Resistance of conifers to invasion by bark beetle-fungus associations. *BioScience*, *22*(10), 598-602.

Blanche, C. A., Lorio, P. L., Sommers, R. A., Hodges, J. D., and Nebeker, T. E. (1992). Seasonal cambial growth and development of loblolly pine: xylem formation, inner bark chemistry, resin ducts, and resin flow. *Forest Ecology and Management*, *49 (1-2)*, 151-165.

Brown, H. D., and McDowell, W. E. (1968). Status of loblolly pine die-off on the Oakmulgee District, Talladega National Forest, Alabama-1968. *US Dep. Agric. For. Serv. Rept*, (69-2), 28.

Chhatre, V. E., Byram, T. D., Neale, D. B., Wegrzyn, J. L., and Krutovsky, K. V. (2013). Genetic structure and association mapping of adaptive and selective traits in the east Texas loblolly pine (*Pinus taeda* L.) breeding populations. *Tree Genet Genomes, 9 (5)*, 1161-1178.

Chieppa, J., Eckhardt, L., and Chappelka, A. (2017). Simulated Summer Rainfall Variability Effects on Loblolly Pine (Pinus taeda) Seedling Physiology and Susceptibility to Root-Infecting Ophiostomatoid Fungi. *Forests*, *8*(4), 104.

Correll, J. C., Gordon, T. R., McCain, A. H., Fox, J. W., Koehler, C. S., Wood, D. L., and Schultz, M. E. (1991). Pitch canker disease in California: pathogenicity, distribution, and canker development on Monterey pine (*Pinus radiata*). *Plant Disease*, *75*(7), 676-682.

Coyle, D. R., Kier, D. K., Frank, H. K., Lawrence, A. M., John, T. N., Steven, W. O., William, J. O., William, D. S., and Kamal, J. K. G. (2015). A review of southern pine decline in North America. *Forest Ecology and Management, 349*, 134-148.

Dwinell, L. D., and Phelps, W. R. (1977). Pitch canker of slash pine in Florida. *Journal of Forestry*, *75*(8), 488-489.

Eckhardt, L. G., and Menard, R. D. (2008). Topographic features associated with loblolly pine decline in central Alabama. *Forest ecology and management, 255(5)*, 1735-1739.

Eckhardt, L. G., Jones, J. P., and Klepzig, K. D. (2004a). Pathogenicity of *Leptographium* species associated with loblolly pine decline. *Plant Disease, 88(11)*, 1174-1178.

Eckhardt, L. G., Menard, R. D., and Gray, E. D. (2009). Effects of oleoresins and monoterpenes on in vitro growth of fungi associated with pine decline in the Southern United States. *Forest Pathology, 39(3)*, 157-167.

Eckhardt, L.G., Goyer, R. A., Klepzig, K. D., and Jones, J. P. (2004b). Interaction of Hylastes species (Coleoptera: Scolytidae) with *Leptographium* species associated with loblolly pine decline. *Journal of Economic Entomology, 97(2)*, 468-474.

Eckhardt, L. G., Weber, A. M., Menard, R. D., Jones, J. P., and Hess, N. J. (2007). Insect-fungal complex associated with loblolly pine decline in central Alabama. *Forest Science, 53(1),* 84-92.

Ferrenberg, S., Kane, J. M., and Mitton, J. B. (2014). Resin duct characteristics associated with tree resistance to bark beetles across lodgepole and limber pines. *Oecologia, 174(4)*, 1283-1292.

Fox, T. R., Jokela, E. J., and Allen, H. L. (2004). The evolution of pine plantation silviculture in the southern United States. Chapter 8.

Gordon, T. R., Okamoto, D., Storer, A. J., and Wood, D. L. (1998). Susceptibility of five landscape pines to pitch canker disease, caused by *Fusarium subglutinans* f. sp. *pini*. *HortScience*, *33*(5), 868-871.

Hammerbacher, A., Schmidt, A., Wadke, N., Wright, L. P., Schneider, B., Bohlmann, J., Brand, W.A., Fenning, T.M., Gershenzon, J., and Paetz, C. (2013). A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce. *Plant physiology, 162(3),* 1324-1336.

Hess, N. J., Otrosina, W. J., Carter, E. A., Steinman, J. R., Jones, J. P., Eckhardt, L. G., Weber A. M and Walkinshaw, C. H. (2002). Assessment of loblolly pine decline in central Alabama.

Jones, C. M., and Hunt, H. E. (1996). Foraging Habitat of the Red-Cockaded Woodpecker on the D'Arbonne National Wildlife Refuge, Louisiana (Habitat de Forrajeo de Picoides borealis en el Refugio Silvestre Nacional D'Arbonne, Louisiana). Journal of Field Ornithology, 511-518.

Joseph, G., Kelsey, R. G., and Thies, W. G. (1998). Hydraulic conductivity in roots of ponderosa pine infected with black-stain (*Leptographium wageneri*) or annosus (*Heterobasidion annosum*) root disease. *Tree Physiology, 18(5)*, 333-339.

Kelley, W. D., and Williams, J. C. (1982). Incidence of pitch canker among clones of loblolly pine in seed orchards. *Plant Disease*, *66*(12), 1171-1173.

Krokene, P., Nagy, N. E., and Solheim, H. (2008). Methyl jasmonate and oxalic acid treatment of Norway spruce: anatomically based defense responses and increased resistance against fungal infection. *Tree Physiology, 28(1),* 29-35.

Manion, P.D. (1981) *Tree Disease Concept*. Prentice-Hall Inc., Englewood Cliffs, NJ.

Matusick, G., and Eckhardt, L. G. (2010). Variation in virulence among four root-inhabiting Ophiostomatoid fungi on *Pinus taeda* L., *P. palustris* Mill, and *P. elliottii* Engelm. seedlings. *Canadian Journal of Plant Pathology, 32(3),* 361-367.

Matusick, G., Eckhardt, L. G., and Enebak, S. A. (2008). Virulence of *Leptographium serpens* on longleaf pine seedlings under varying soil moisture regimes. *Plant Disease, 92(11),* 1574-1576.

Matusick, G., Somers, G., and Eckhardt, L.G. (2012). Root lesions in large loblolly pine (*Pinus taeda* L.) following inoculation with four root-inhabiting ophiostomatoid fungi. *Forest Pathology, 42(1)*, 37-43.

Matusick, G., Eckhardt, L. G., and Somers, G. L. (2010). Susceptibility of longleaf pine roots to infection and damage by four root-inhabiting ophiostomatoid fungi. *Forest Ecology and Management, 260(12),* 2189-2195.

Matusick, G., Nadel, R. L., Walker D. M., Hossain M. J., and Eckhardt, L. G. (2016). Comparative behavior of root pathogens in stems and roots of southeastern Pinus species. *Fungal Biology, 120(4)*, 471-480.

Oliva, J., Stenlid, J., and Martínez-Vilalta, J. (2014). The effect of fungal pathogens on the water and carbon economy of trees: implications for drought-induced mortality. New Phytologist, 203(4), 1028-1035.

Otrosina, W. J., Bannwart, D., and Roncadori, R. W. (1999). Root-infecting fungi associated with a decline of longleaf pine in the southeastern United States. *Plant and Soil, 217(1)*, 145-150.

Otrosina, W. J., Walkinshaw, C. H., Zarnoch, S. J., Sung, S., and Sullivan, B. T. (2000). Root disease, longleaf pine mortality, and prescribed burning. *In Proceedings of the eleventh biennial southern silvicultural research conference.* U.S. Dep. Agric. For. Serv. Gen. Tech. Rep. SRS-48. Asheville, NC.

Paine, T. D. (1984). Seasonal response of ponderosa pine to inoculation of the mycangial fungi from the western pine beetle. *Canadian Journal of Botany, 62(*3), 551-555.

Paine, T. D., Raffa, K. F., and Harrington, T. C. (1997). Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Annual review of entomology, 42(1),* 179-206.

Popp, M. P., Johnson, J. D., and Lesney, M. S. (1995). Changes in ethylene production and monoterpene concentration in slash pine and loblolly pine following inoculation with bark beetle vectored fungi. *Tree Physiology, 15*, 807-812.

Rauscher, H. M. (2004). A history of southern forest science, management, and sustainability issues. Gen. Tech. Rep.USDA Forest Service, Southern Research Station. Chapter 1, 3-4.

Rice, A. V., Thormann, M. N., and Langor, D. W. (2007a). Mountain pine beetle associated blue-stain fungi cause lesions on jack pine, lodgepole pine, and lodgepole× jack pine hybrids in Alberta. *Botany*, *85*(3), 307-315.

Rice, A. V., Thormann, M. N., and Langor, D. W. (2007b). Virulence of, and interactions among, mountain pine beetle associated blue-stain fungi on two pine species and their hybrids in Alberta. *Botany*, *85*(3), 316-323.

Roux, J., Eisenberg, B., Kanzler, A., Nel, A., Coetzee, V., Kietzka, E., and Wingfield, M. J. (2007). Testing of selected South African Pinus hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. *New Forests*, *33*(2), 109-123.

Schultz, R. P. (1999). Loblolly—the pine for the twenty-first century. In Planted Forests: Contributions to the Quest for Sustainable Societies (pp. 71-88). Springer Netherlands.

Schultz, R. P. (1997). *Loblolly pine: the ecology and culture of loblolly pine* (*Pinus taeda* L.). Agriculture Handbook. USDA Forest Service, Washington D.C.

Schultz, M. E., Gordon, T. R., and McCain, A. H. (1990). Resistance of Monterey pine (Pinus radiata) to pitch canker disease caused by Fusarium subglutinans. *Phytopathology*, *80*, 977.

Singh, A., Anderson, D., and Eckhardt, L. G. (2014). Variation in resistance of loblolly pine (*Pinus taeda* L.) families against *Leptographium* and *Grosmannia* root fungi. *Forest Pathology, 44(4),* 293-298.

Stephen, F. M., and Paine, T. D. (1985). Seasonal patterns of host tree resistance to fungal associates of the southern pine beetle. *Zeitschrift für angewandte Entomologie*, 99(1-5), 113-122.

Tyree, M. T., and Zimmermann, M. H. (2002). *Xylem Structure and the Ascent of Sap*, Springer. Berlin, Germany.

Westbrook, J. W., Resende, M. F., Munoz, P., Walker, A. R., Wegrzyn, J. L., Nelson, C. D., Neale, D. B., Kirst, M., Huber, D. A., Gezan, S. A., Peter, G. F., and Davis, J. M. (2013). Association genetics of oleoresin flow in loblolly pine: discovering genes and predicting phenotype for improved resistance to bark beetles and bioenergy potential. *New Phytologist, 199(1),* 89-100.

Yadeta, K., Thomma, B. 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. *Frontiers in Plant Science* 4: art no. 97.

Figure 1.1 Two primary lateral roots of *Pinus taeda* excavated for the fungal inoculation.

Figure 1.2 Inoculation of the agar plug with fungi in the lateral root of *Pinus taeda.*

Figure 1.3 Re-excavation of the fungi inoculated lateral roots of *Pinus taeda* for further measurements.

Figure 1.4 *Pinus taeda* root samples ready for measurement in the laboratory.

Figure 1.5 Resinosis and necrosis in the root 8 weeks following inoculation with *Leptographium terebrantis* in family S2.

Figure 1.6 Wound control treated root section with small necrotic area.

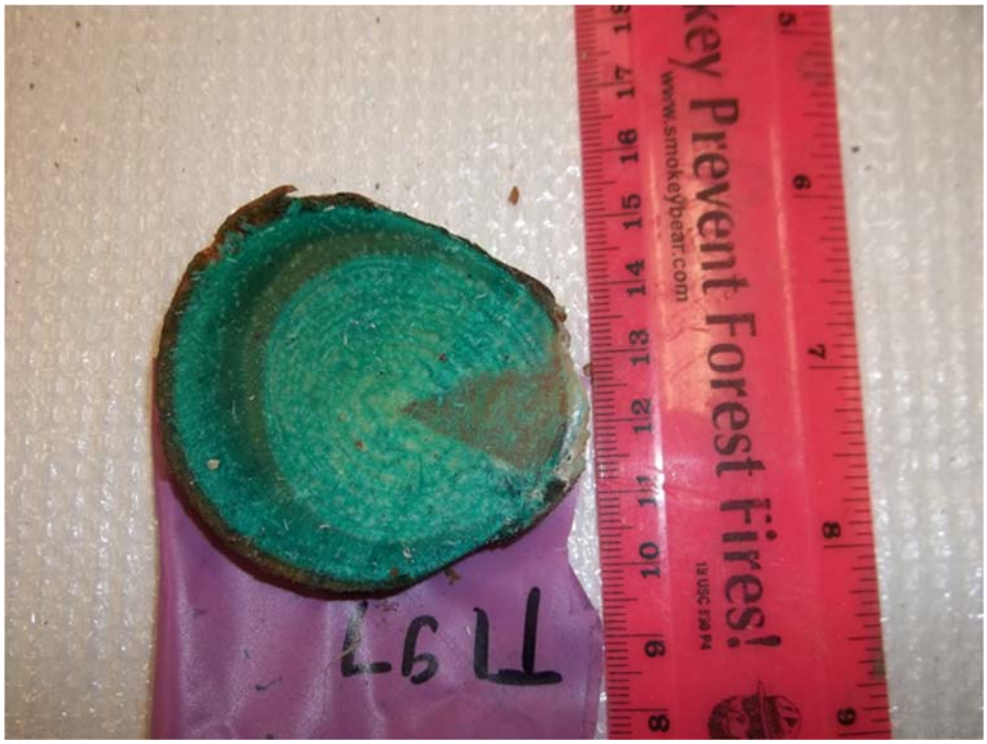

Figure 1.7 Pie-shaped occlusion observed at the cross-section of inoculated root.

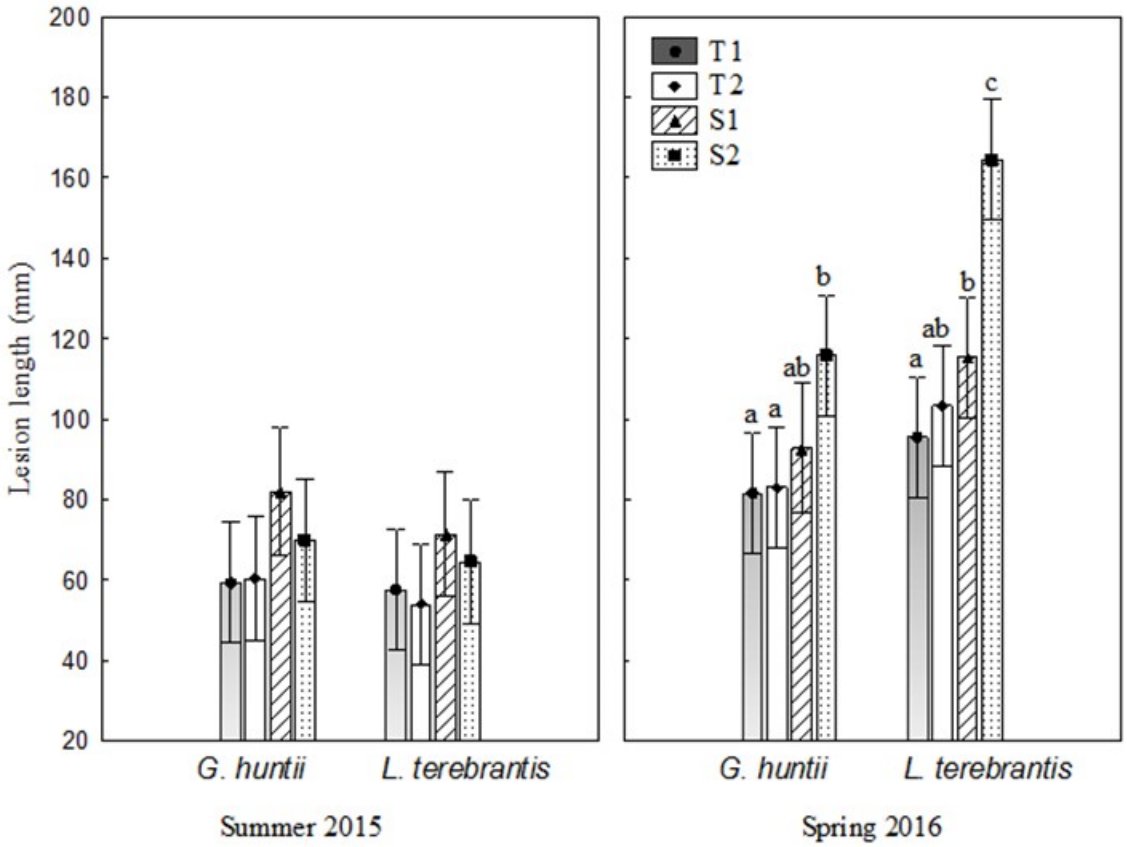

Figure 1.8 Lesion length caused by the two different fungi in summer 2015 and spring 2016 inoculations. T1 and T2: Families tolerant to root-infecting ophiostomatoid fungi at seedling stage. S1 and S2: Families susceptible to root-infecting fungi and seedling stage. 95% confidence intervals are indicated by error bars.

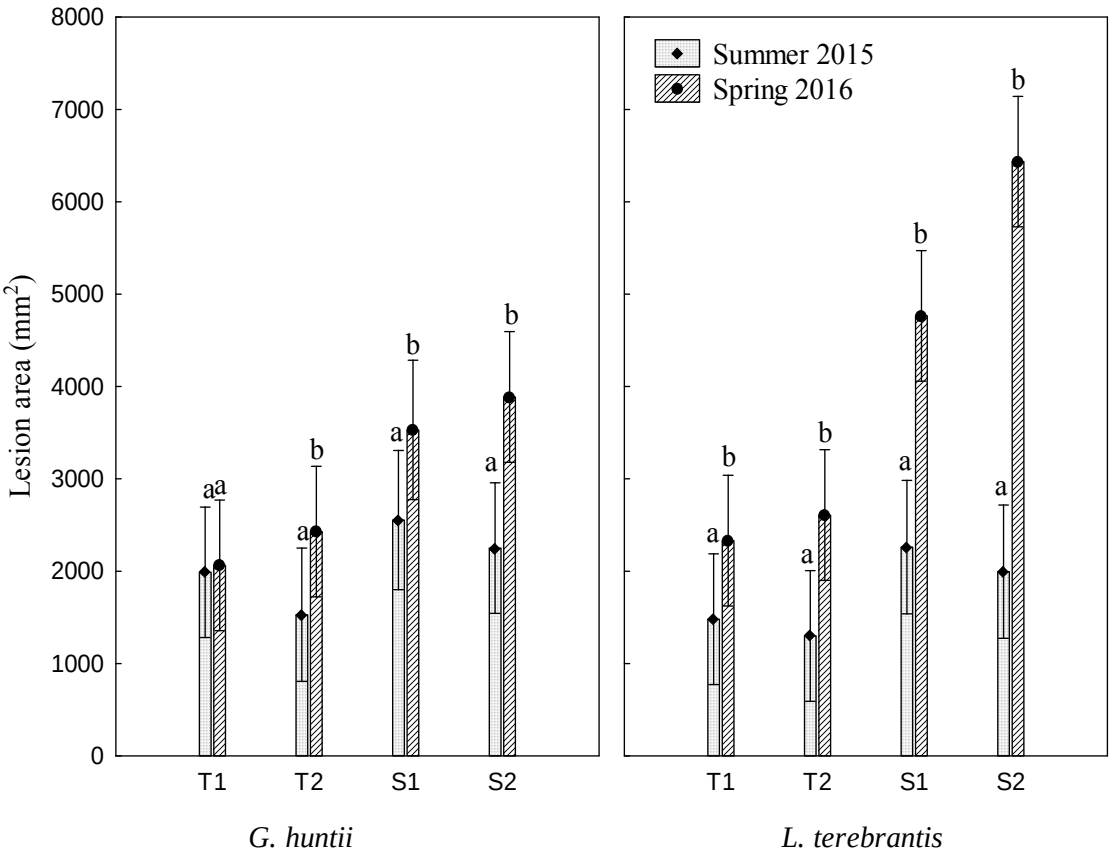

Figure 1.9 Lesion area caused by the two different fungi in summer 2015 and spring 2016 inoculations. T1 and T2: Families tolerant to root-infecting ophiostomatoid fungi at seedling stage. S1 and S2: Families susceptible to root-infecting ophiostomatoid fungi at seedling stage. 95% confidence intervals are indicated by error bars.

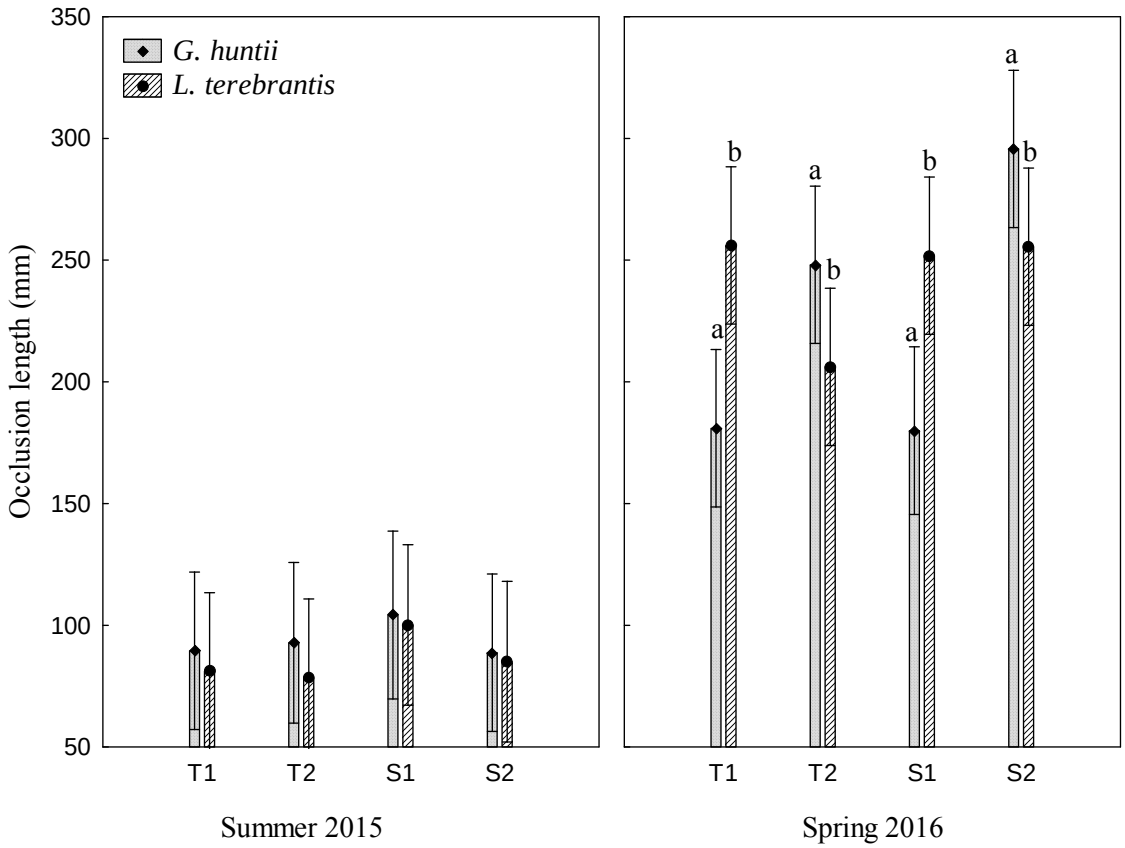

Figure 1.10 The length of occluded tissue produced by the two different fungi in summer 2015 and spring 2016 inoculations. T1 and T2: Families tolerant to root-infecting fungi. S1 and S2: Families susceptible to root-infecting ophiostomatoid fungi at seedling stage. 95% confidence intervals are indicated by error bars.

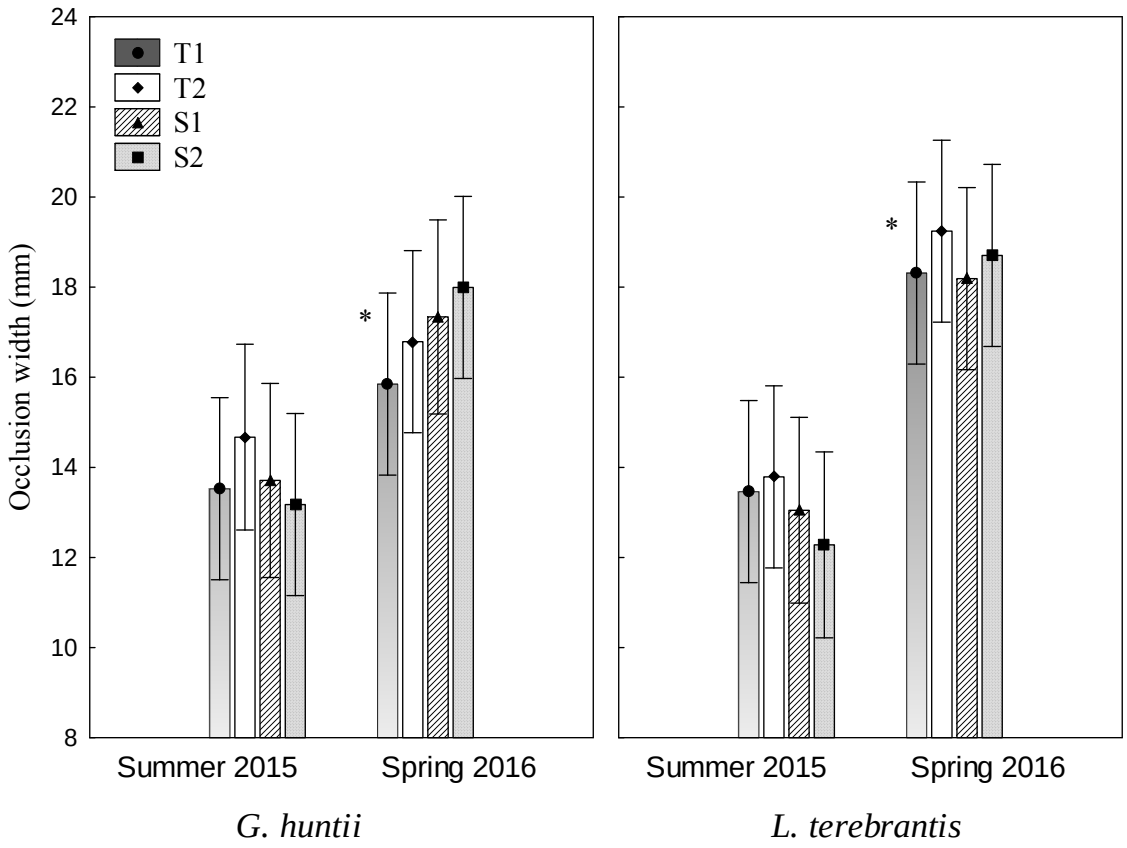

Figure 1.11 The width of occluded tissue produced by the two different fungi in summer 2015 and spring 2016 inoculations. T1 and T2: Families tolerant to root-infecting fungi. S1 and S2: Families susceptible to root-infecting ophiostomatoid fungi and seedling stage. 95% confidence intervals are indicated by error bars.

Table 1.1 P-values for fixed effect and possible interactions for summer 2015 and spring 2016 inoculation.

Time	Variable	DF	Lesion Area	Lesion Length	Lesion Width	Occlusion Length	Lesion Depth	Occlusion Width	Occlusion Depth
2015	RD	1	0.01	0.01	0.01	0.08	0.65	<0.0001	0.02
	Fam	3	<0.0001	0.31	0.31	0.03	0.12	0.10	<0.0001
	Trt	1	0.08	0.10	0.10	0.10	0.58	0.34	0.02
	Fam*trt	3	0.85	0.46	0.46	0.86	0.38	0.94	0.51
2016	RD	1	0.03	0.86	<0.0001	0.99	<0.0001	<0.0001	<0.0001
	Fam	3	<0.0001	<0.0001	<0.0001	0.03	0.02	0.43	0.12
	Trt	1	0.002	<0.0001	0.62	0.31	0.31	0.10	<0.0001
	Fam*Trt	3	0.03	0.32	0.54	0.01	0.37	0.73	0.29

(Note: RD: Root-diameter, Fam: Family, Trt: Fungal treatment). P-values were estimated at $\alpha = 0.05$.

Table 1.2 Familywise mean and standard deviation of all the response variables for 2015 summer inoculation.

Family	N	Lesion Area (mm^2)	Lesion Length (mm)	Lesion Width (mm)	Occlusion Length (mm)	Lesion Depth (mm)	Occlusion Width (mm)	Occlusion Depth (mm)
T1	50	1734.44^{ab}	58.50^{a}	38.46^{a}	85.34^{a}	3.64^{a}	13.49^{a}	6.81^{a}
		(1010.62)	(18.62)	(15.51)	(24.90)	(1.43)	(3.00)	(3.02)
T2	49	1411.59^{b}	57.02^{ab}	37.86^{a}	85.54^{a}	3.96^{a}	14.22^{a}	7.11^{a}
		(635.08)	(12.46)	(12.33)	(29.08)	(1.54)	(2.45)	(3.08)
S1	46	2400.76^{c}	76.41^{c}	43.13^{a}	102.09^{b}	3.35^{a}	13.37^{a}	5.54^{b}
		(1553.76)	(28.38)	(12.22)	(38.42)	(0.96)	(3.68)	(2.67)
S2	49	2125.22^{ac}	67.23^{ac}	38.38^{a}	86.96^{a}	5.84^{b}	12.74^{a}	5.42^{b}
		(1255.88)	(22.66)	(16.04)	(25.65)	(10.98)	(4.08)	(2.86)

T1 and T2: Families considered tolerant to root-infecting ophiostomatoid fungi at seedling stage, and S1 and S2: Families considered susceptible root-infecting ophiostomatoid fungi at seedling stage. Means followed by the standard deviation in parenthesis. Numbers followed by same letters within each column are not significantly different at $\alpha = 0.05$.

Table 1.3 Parameter estimates of the response variables between the families in summer 2015 inoculation.

	Lesion Area (mm^2)		Lesion Length (mm)		Occlusion Length (mm)		Occlusion Depth (mm)	
Family	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value
T1 vs T2	419.78	0.07	2.67	0.53	1.49	0.80	-0.08	0.89
	(230.30)		(4.27)		(6.10)		(0.58)	
T1 vs S1	-545.09	0.02	-16.41	0.0002	-14.63	0.01	1.54	0.01
	(235.31)		(4.37)		(6.16)		(0.59)	
T1 vs S2	-305.25	0.18	-7.67	0.07	-0.13	0.98	1.59	0.007
	(229.76)		(4.26)		(6.01)		(0.58)	
T2 vs S1	-964.87	<0.0001	-19.09	<0.0001	-16.12	0.008	1.62	0.006
	(232.88)		(4.32)		(5.99)		(0.57)	
T2 vs S2	-725.02	0.002	-10.34	0.02	-1.62	0.78	1.66	0.004
	(229.04)		(4.25)		(6.10)		(0.58)	
S1 vs S2	239.84	0.30	8.74	0.04	-14.50	0.01	0.04	0.94
	(233.05)		(4.33)		(6.09)		(0.58)	

T1 and T2: Families considered tolerant to root-infecting ophiostomatoid fungi at seedling stage, and S1 and S2: Families considered susceptible to root-infecting ophiostomatoid fungi at seedling stage. Estimates followed by the standard error in parenthesis. P-values show significant differences at $\alpha = 0.05$.

Table 1.4 Means of response variables in four families inoculated with *L. terebrantis* and *G. huntii* in spring 2016.

Fungi	Family	N	Lesion Area (mm^2)	Lesion Length (mm)	Lesion Width (mm)	Occlusion Length (mm)	Occlusion Depth (mm)
LT	T1	25	2330.88[a]	95.44[a]	33.24[a]	256.04[a]	12.23[a]
			(729.69)	(31.40)	(5.02)	(81.64)	(5.22)
	T2	25	2608.20[a]	103.26a[b]	37.86[a]	206.20[a]	16.35[a]
			(847.55)	(26.31)	(13.53)	(83.11)	(8.31)
	S1	25	4764.84[b]	115.31a[b]	50.41[a]	251.84[a]	14.96[a]
			(2126.54)	(41.78)	(17.83)	(104.67)	(5.93)
	S2	25	6435.40[c]	164.520[c]	53.79[a]	255.54[a]	14.11[a]
			(4610.77)	(96.08)	(22.2)	(95.32)	(5.71)
GH	T1	25	2063.64[a]	81.59[a]	34.97[a]	180.95[a]	10.04[a]
			(791.27)	(21.57)	(12.71)	(90.84)	(3.89)
	T2	25	2429.40[a]	83.01[a]	38.07[b]	248.07[b]	9.70[a]
			(1443.48)	(34.94)	(10.40)	(106.15)	(3.47)
	S1	22	3529.73[b]	92.71[a]	45.85[b]	179.96[ab]	13.83[b]
			(2151.41)	(37.39)	(19.89)	(101.89)	(9.15)
	S2	25	3886.36[b]	115.75[c]	50.52[c]	295.69[c]	10.13[ab]
			(2524.02)	(62.44)	(13.28)	(190.79)	(3.89)

Note: LT: *Leptographium terebrantis*, and GH: *Grosmannia huntii*. T1 and T2: Families considered tolerant to root-infecting ophiostomatoid fungi at seedling stage, and S1 and S2: Families considered susceptible to root-infecting ophiostomatoid fungi at seedling stage. Means followed by the standard deviation in parenthesis. Numbers followed by same letters within each column within each fungus are not significantly different at $\alpha = 0.05$.

Table 1.5 Estimate of difference in response variables between families inoculated with *L. terebrantis* and *G. huntii* in spring 2016.

		Lesion Area (mm^2)		Lesion Length (mm)		Lesion Width (mm)		Occlusion Length (mm)		Occlusion Depth (mm)	
Fungi	Family	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value
LT	T1 vs T2	-129.41	0.86	-10.27	0.53	-1.30	0.77	39.73	0.14	-0.73	0.53
		(758.05)		(16.43)		(4.44)		(26.47)		(1.16)	
	T1 vs S1	-25359.26	0.002	-21.10	0.19	-15.50	0.0006	-0.91	0.97	-1.03	0.37
		(742.07)		(16.08)		(4.35)		(25.90)		(1.13)	
	T1 vs S2	-4095.43	<0.0001	-69.23	<0.0001	-20.34	<0.0001	-0.11	0.99	-1.69	0.14
		(736.60)		(15.96)		(4.32)		(25.71)		(1.13)	
	T2 vs S1	-2229.84	0.003	-10.82	0.50	-14.20	0.0015	-40.63	0.12	-0.30	0.79
		(741.85)		(16.06)		(4.35)		(25.90)		(1.13)	
	T2 vs S2	-3966.01	<0.0001	-58.95	0.0005	-19.04	<0.0001	-39.84	0.13	-0.96	0.41
		(755.51)		(16.37)		(4.43)		(26.38)		(1.15)	
	S1 vs S2	-1736.17	0.02	-48.12	0.004	-4.85	0.27	0.79	0.98	-0.66	0.56
		(740.80)		(16.05)		(4.34)		(25.86)		(1.13)	
GH	T1 vs T2	-333.94	0.51	-1.07	0.93	-2.70	0.46	-66.07	0.07	0.44	0.76
		(501.45)		(11.81)		(3.61)		(36.49)		(1.47)	
	T1 vs S1	-1344.11	0.01	-9.81	0.43	-9.37	0.01	5.12	0.90	-3.39	0.03
		(519.85)		(12.25)		(3.74)		(37.83)		(1.53)	
	T1 vs S2	-1952.93	0.0002	-35.55	0.004	-17.17	<0.0001	-119.15	0.001	-0.51	0.73
		(503.35)		(11.86)		(3.63)		(36.63)		(1.48)	
	T2 vs S1	-1011.17	0.05	-8.75	0.48	-6.68	0.07	-71.12	0.06	-3.84	0.01
		(519.50)		(12.23)		(3.74)		(36.77)		(1.53)	
	T2 vs S2	-1619.99	0.002	-34.50	0.005	-14.76	0.0001	-53.13	0.15	-0.96	0.52
		(504.50)		(11.88)		(3.64)		(36.71)		(1.48)	
	S1 vs S2	-608.82	0.25	-25.74	0.04	-7.80	0.04	-124.26	0.002	2.88	0.07
		(525.46)		(12.40)		(3.79)		(38.23)		(1.54)	