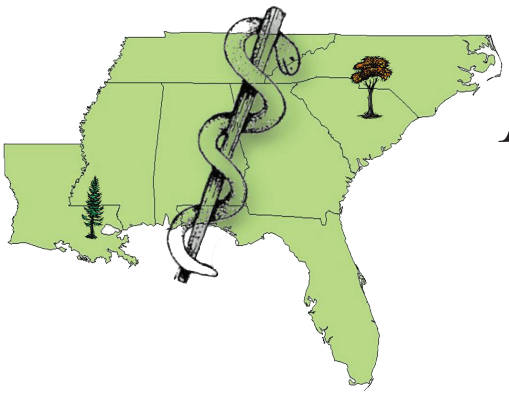

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VARIATION IN RESISTANCE OF LOBLOLLY PINE (PINUS TAEDA L.) AND SLASH PINE (P. ELLIOTII ENGLEM.) FAMILIES AGAINST LEPTOGRAPHIUM AND GROSMANNIA ROOT FUNGI: YEAR ONE RESULTS

by
Amrit Singh and Lori Eckhardt

ABSTRACT
Pine decline poses a serious threat to forest sustainability in the southeastern United States. Complex interactions of biotic and abiotic factors are involved that include root-feeding bark beetle vectors and their associated fungal genera *Leptographium* and *Grosmannia*. A screening study was conducted to determine the relative resistance of loblolly (*Pinus taeda* L.) and slash pine (*P. elliottii* Englem.) seedling families when challenged with *Leptographium* and *Grosmannia*. Bare root seedlings from loblolly and slash pine families (23 and 5 respectively) were screened for resistance using an artificial inoculation method. Seedling responses such as lesion presence, lesion length, occlusion of vascular tissues and seedling survival were measured twelve weeks after inoculations. Seedling stems exhibited dark brown lesions and resinous occluded tissues. Results indicated that the seedling families had variable responses to different *Leptographium* and *Grosmannia* species leading to identification of some families (L-5, L-20, L-8, and, L-13) that developed consistently smaller lesions, while other families (L-1, L-2, L-3 and L-4) were found to have consistently larger lesions. These responses indicate that family genetics could be used for deployment into high risk areas to mitigate the potential for pine decline.

INTRODUCTION
The diverse and dynamic nature of southeastern forests is the result of the variations in land use history (Wear and Greis 2002). Before European settlements, the southeastern United States was under natural forest conditions with mixed and sparse tree stands consisting of oak- pine canopy and naturally occurring fires controlling the understory vegetation. In the 19th and early 20th centuries there were large-scale disturbances in forest ecosystems throughout the region. The forests were cleared for agriculture and cultivation of row crops led to soil erosion and nutrient depletion. Agriculture was abandoned but the problem of erosion persisted due to the absence of vegetation. With the initiation of Federal and State agencies, loblolly pine (*Pinus taeda* L.) was widely planted in the region to prevent further soil erosion. This particular pine species exhibits rapid juvenile growth; responds well to silvicultural treatments and can be easily established (Baker and Langdon 1990).

Since 1953, there has been a continuous increase in pine plantations in the southeastern United States

(Haynes et al. 2007). At present, large portions of the southeastern United States forests are managed for wood products, including pulp, saw timber and poles. Loblolly and slash pine are two important tree species grown in this region. Loblolly pine continues to be the most favored species for timber production in the Southeast consisting of 80% commercial forest area in loblolly pine (Smith et al. 2007). The forest industry within the southeastern United States provides 110,000 jobs directly or indirectly and contributes $30 billion to the economy of the region. Beside timber production, loblolly pine offer a variety of other environmental related benefits like soil erosion control, habitat for wildlife, landscape and recreational activities (Schultz 1999).

Recently, southern pine decline or premature mortality have been observed and characterized as a decline syndrome (Eckhardt et al. 2007). The decline concept was first described by Manion (1981). Tree decline diseases involve a number of factors that together contribute to mortality and these factors have been broadly classified as predisposing, inciting and contributing. Predisposing factors bring the tree under constant stress. Inciting factors act for a short period of time and increase the severity of the stress and contributing factors adds to decline in the end, resulting in premature mortality. Decline in loblolly pine was first noticed in 1959 in the Talladega National Forest on the Oakmulgee and Tuscaloosa Ranger districts in Alabama (Brown and McDowell 1968). At that time, the premature tree mortality was coined “loblolly pine die-off”. Loblolly pine is host to a number of beetle species that vector the fungi associated with pine decline. There is a complex interaction of these insects and fungi that together contribute to the observed pine decline (Eckhardt et al. 2007). Among the biotic factors, *Leptographium* has a contributing role in pine decline. Earlier, several attempts were made to identify the fungi associated with decline. Potential root pathogens studied were *Phytophthora cinnamomi* Rands., *Heterobasidium irregulare* nom. nov. Garbelotto & Otrosina and *Leptographium* spp. It is now well understood that ophiostamatoid fungi are an important component of pine decline (Hess et al. 2005; Eckhardt et al. 2007; Eckhardt and Menard 2009). Earlier ophiostomatoid root fungi have caused significant damage in many pine ecosystems. *Leptographium wageneri* (W.B. Kendr.) M.J. Wingf, was found to be associated with black stain root disease in conifers of northwestern United States (Cobb 1988). *Leptographium procerum* (Kendrick) M. J. Wingfield, was recovered from the roots and stumps of slash pine plantations (Barnard et al. 1991). Among others, red pine (*P. resinosa* Ait*)* decline in Wisconsin (Klepzig et al. 1991), eastern white pine (P. *strobus* L.) decline (Dochinger 1967) and *Leptographium* spp. recovery from the southern pine plantations under SPB attack (Otrosina et al. 1997) are a few examples.

Several studies have focused on testing the relative virulence of the *Leptographium* species to pine (Wingfield 1983; Harrington and Cobb 1983; Eckhardt et al. 2004a; Matusick and Eckhardt 2010a). *Leptographium procerum* and *L. terebrantis* S. J. Barras & T. J. Perry are well studied and have been included in several virulence studies. *Leptographium procerum* was found causing procerum root disease in eastern white pine (Dochinger 1967). However, in other virulence studies *L. procerum* was found to be a mild pathogen. *Leptographium procerum* did not cause significant damage, while *L. terebrantis* caused extensive mortality to white pine seedlings (Wingfield 1983). *Leptographium procerum* was found to be less virulent to southern pine species than other *Leptographium* species commonly found associated with southern pines (Eckhardt et al. 2004a; Matusick and Eckhardt 2010a). *Leptographium terebrantis* is considered

to be mild to moderate pathogen. It has consistently produced resin soaked lesions (Nevill et al. 1995) and sapwood discoloration in pines (Matusick and Eckhardt 2010b). *Leptographium terebrantis* was frequently isolated from the study seedlings of ponderosa pine (*P. ponderosa* P. & C. Lawson) and Douglas-fir (*Pseudotsuga* spp.) but was not linked to black-stain root disease (Harrington and Cobb 1983).

Among other species of *Leptographium, G. alacris* (formely *L. serpens*) T.A. Duong, Z.W. de Beer & M.J. Wingf. sp. nov., and *Grosmannia huntii* (R. C. Rob. Jeffr.) have been found to be associated with southern pines. *Grosmannia alacris* has been recovered from loblolly pine roots infested with *H. salebrosus* and *H. tenius* insect vectors (Eckhardt et al. 2007). *Grosmannia alacris* along with *L. lundbergii* produced the longest lesions in a study conducted on loblolly pine seedlings and mature trees (Eckhardt et al. 2004a). *Grosmannia alacris* and two other *Leptographium* species (*Ophiostoma ips* and *L. lundbergi*) were not considered serious pathogens of pine forests in South Africa based on the virulence trials (Zhou et al. 2002). Morphological distinction between *G. alacris* and *G. huntii* was difficult with both species having distinctive serpentine hyphae. Recently, the presence of *G. huntii* in the southeastern United States was confirmed using DNA sequence data (Zanzot 2009) and has been recovered from longleaf pine (*P. palustris* Mill.) plantations in Georgia (Zanzot et al. 2010). Virulence of *G. huntii* has been tested recently on southern pine species and it resulted in the longest lesions in all the three pine species included in the study (Matusick and Eckhardt 2010a).

Previous studies have been focused on testing the relative virulence of *Leptographium* root fungi on a particular pine species (Wingfield 1983; Harrington and Cobb 1983; Eckhardt et al. 2004a; Bertagnole et al. 1983; Nevill 1995). Recently, the relative virulence of the *Leptographium* root fungi and comparative resistance/tolerance of the three main southern pine species have been studied and it has been established that different *Leptographium* species vary in their virulence among southern pines. Also, longleaf pine followed by slash pine was found to be more tolerant to *Leptographium* than loblolly pine (Matusick and Eckhardt 2010a).

Loblolly and slash pine are prone to a wide variety of insect pests and diseases that include fusiform rust (*Cronartium quercum* [Berk.] Miyabe. Ex Shirai. f. sp. *fusiforme*). Resistance to fusiform rust has been incorporated into genetically improved seedlings by tree breeding efforts along with increases in growth rates. As a result of these breeding programs the infection rate of fusiform rust has been decreased to 20-25% in the improved seedlots (Li et al. 1999). However, studies have yet to focus on the relative virulence of the *Leptographium* species within commonly deployed pine families of either loblolly or slash pine. These studies are the first to look into within species variability for resistance to *Leptographium* root fungi among the loblolly pine and slash pine families deployed by forest industries throughout the southeastern United States.

MATERIALS AND METHODS

Seed from 28 families (23 loblolly and 5 slash) representing some of the more commonly out-planted genotypes, were grown in a common nursery bed at the Glennville Regeneration center in Georgia. Seedlings were lifted in early January 2011 and re-potted at Auburn University in an outdoor screening facility. Trade gallon pots filled with ProMix BX® (Premier Tech, Quebec, and Canada) peat based potting media were used for planting the seedlings. To maintain

anonymity each family was assigned a unique code for data reporting and analysis. For both the years, seedlings were placed under natural environmental conditions with access to natural precipitation, sunlight and temperature with irrigation to prevent moisture stress. The experiment was set up as a randomized complete block design with three blocks/replications in first and second year. Pre-treatment and final (just before harvesting) root collar diameter and height measurements were taken to monitor growth. To determine the pre-pot family morphology, in year two, ten seedlings per family were subsampled before re-potting and after root collar diameter and height measurements, each seedling was separated into fine roots, coarse roots, stem and foliage. These parts were oven dried separately to calculate the dry biomass. Initial root to shoot ratio was calculated from the dry weight measurements.

Inoculation treatments were applied two months after re-potting the seedlings. Six treatments were assigned to randomly selected seedlings within each family. Ten seedlings per family per block were selected for each treatment. The treatments included inoculations with one of four fungal species: *L. procerum*, *L. terebrantis*, *G. alacris* and *G. huntii*. A wounded and wound+media served as the controls. Two weeks before inoculations, each fungus was cultured on 2% malt extract agar (MEA). Pure isolates of the fungal species were used for inoculations. Wound inoculation method included making a vertical cut with sterile razor blade in the lower stem of the seedling about 2 cm from the soil line (Nevill et al. 1995; Eckhardt et al. 2004a) (Figure 1). A 3 mm diameter plug of colonized mycelium was placed in the slit and the wound was wrapped with moist cotton and sealed with Parafilm®.

After destructively harvesting the seedlings, final seedling growth measurements and seedling survival were recorded. On each seedling stem, seedling responses to the fungal inoculations were measured. These included lesion presence/absence, lesion length, lesion width, lesion depth and occlusion of vascular tissues. Lesion length consisted of dark brown tissue which either equals or extends to both sides of the length of the inoculation wound. For determining occlusion length, the roots were separated from the shoots and the living shoots were placed in a solution of FastGreen stain (FastGreen FCF; Sigma Chemical Co.) and water (0.25g/L of water) (Nevill et. al. 1995) (Figure 2). After three days of allowing capillary action, the length of unstained stem tissue was recorded as occlusion length. To confirm if the fungal infection occurred with the inoculations, re-isolation of associated fungi was attempted by cutting 1 cm of stem section surrounding the lesion and plating it on CSMA nutrient media (Malt extract agar with 800 mg/l of cycloheximide and 200 mg/l of streptomycin sulfate).

Seedling response variables to the artificial inoculations were analyzed using SAS statistical software (SAS Institute, 9.2 ed., Cary, NC). Both generalized linear fixed model and linear mixed models were fit to the data to compare the treatments and families. In the fixed model, each block was considered a replicate. The variables families, fungal treatments and their interaction were included in the model under fixed effects. A set of ten seedlings per family in each replication was used as an experimental unit for all the continuous response variables while running the analysis for year one screening. Lesion length was found to be the strongest response variable. For comparison among the pine families, lesion length was used as a response variable. Families were assigned into groups based on their average lesion length and analysis was conducted using contrast statements to find significant differences between the groups. The contrast statements were used on the main effects and interaction effects. Binary response

variables like survival, lesion presence and re-isolation were analyzed in GENMOD procedure in SAS using logistic ANOVA for the second year screening as it was noticed that unlike the first year, mortality was found to be significant.

In the mixed model, family and family x treatment interaction were kept random, while the treatments were kept under fixed effects. All the continuous variables were analyzed considering the seedling as an experimental unit. Both main effects and two way interaction effects were included in the model. Covariance parameter estimates for each response variable such as lesion length, occlusion length, lesion width and lesion depth were used to determine the variation among the families and the families were ranked on the basis of covariance parameter estimates for lesion length. Fungal treatments were compared from type 3 fixed effects.

RESULTS

Inoculation of the 28 different pine families with the 4 different fungi resulted in less than 6.5% seedling mortality. A total of 93.5% of the seedlings survived the inoculations. Seedling mortality was observed in families L-14 and L-22 which had comparatively less survival than the other loblolly pine families tested. All fungi used in the trial caused dark brown lesions along seedling stems. Lesions extended vertically beyond the inoculation site along both sides of the seedling stem (Figure 3). Evidence of radial movement of the fungus was observed more so in seedlings challenged with *G. huntii.* As a comparison, lesions did not occur beyond the inoculation site in either of the wound or wound+media controls and they were not significantly different from each other ($F = 0.07$, $P = 0.8231$). However, lesion length was found to be significantly longer for all four fungi tested compared to the two control treatments (Table 1). While lesions occurred in all seedlings inoculated with the fungal species, their presence as indicated from the fixed model results was not significantly different among the fungal treatments and families (Table 2).

Of the 6 seedling variables measured, lesion length was considered the primary seedling response variable. Average lesion length was found to be significantly different among the families ($F=2.87$, $P=0.0001$) and among the fungi tested ($F=49.56$, $P=<0.0001$) (Table 2). Average lesion length was not significantly different among the fungal treatments within each family ($F=0.60$, $P=0.9878$) (Table 2). Similar to lesion length, occlusion length was also significantly different among the families ($F=2.63$, $P= 0.0004$) (Table 2) and among the fungi ($F=57.60$, $P=<0.0001$) (Table 2) but not within each family ($F=0.84$, $P=0.744$) (Table 2). When average lesion length was plotted for each fungus separately, it was observed that seedlings inoculated with *G. huntii* formed three distinct family groups with the intermediate group not significantly different from either extreme group (Figure 4). When seedlings were challenged with *G. alacris*, families L-1, L-2, L-3, L-4, and L-21 were found to have significantly longer lesions than all other families examined (Figure 5). Likewise, when *L. terebtantis* was used, the group of families with intermediate lesion length was found to be significantly different from both the extreme groups having smallest and largest lesion length (Figure 6). There was no difference in lesion length among families tested when *L. procerum* was used (Figure 7). However, the overall family ranking trend of *L. procerum* was found to be similar to the other three fungal species. With respect to the other seedling characteristics, while the RCD was found to be different among the families tested, the final mean root collar diameter (RCD) was not

affected by the fungal inoculations. Similar to mean RCD, mean height was also not affected by the fungal inoculations but it varied significantly among different families (Table 3).

The interaction (family*fungi) was not significant for lesion and occlusion length, indicating that the four fungi used in the inoculations did not vary in their virulence within a particular seedling family. Therefore, the 23 loblolly pine families were ranked for their overall response to the artificial inoculations by pooling the fungal species together (Figure 5). Group wise comparison using average lesion length among the seedling families sorted the loblolly pine families into three distinct groups. Family response (lesion length) to the fungal inoculations within each group was found to be different from the other two groups (Figure 5). In the slash pine families, fungal species used significantly affected both the lesion and occlusion length overall (F=16.09, P=<0.0001) (Table 4). One slash pine family S-1 had significantly longer lesions than families S-3 and S-5 caused by *L. terebrantis* (Figure 6). No other differences were observed on slash pine families for other variables measured: lesion and occlusion presence or lesion and occlusion length. Average lesion length, occlusion length, percentage survival and percentage re-isolation for loblolly pine families and slash pine families are shown in Table 5 and 6 respectively.

Lesions did not extend beyond the inoculation site in either of the wound or wound+media controls and were not significantly different from each other (F = 0.06, P = 7990). This was further supported by the mixed model results from pairwise comparison of the treatments (Table 7). Further analysis was completed with four fungal treatments. Estimation of covariance parameters as presented in Table 8 indicated that the variation among the families was significantly different from zero for lesion length, and occlusion length. However, lesion width and lesion depth was not significantly different from zero for the loblolly pine families tested (Table 8). Type 3 fixed effects suggested that the fixed effects including blocks and treatments were significantly different for lesion length, occlusion length, lesion width and lesion depth (Table 9). Family x treatment interaction was found to be significant and the families were ranked overall and for each fungal species using lesion length on the basis of the estimate (i.e. mean family performance) (Table 10). Family ranking differed overall and for each treatment. As an example, family L-20 ranked first overall, second for *L. terebrantis*, fourth for *G. alacris* and twenty second for *L. procerum* (Table 10).

DISCUSSION

In the year one seedling family screening experiment, inoculation with the four different fungal species was able to identify loblolly and slash pine families that would be considered either more or less resistant to the interactions of the root-feeding bark beetles and the introduction of their associated fungi into these widely planted genotypes (Figures 8 & 9). All four fungal species successfully infected pine seedlings as lesions formed on all families of loblolly and slash pine. It is obvious that the lesions did not extend the length of inoculation wound in several cases. While the virulence of ophiostomatoid fungi has been tested in previous studies (Nevill et al. 1995; Eckhardt et al. 2004a; Matusick and Eckhardt 2010a), these inoculation trials were the first to examine within species variability of pine families for resistance against *Leptographium* and *Grosmannia* species. Differences in the relative virulence of fungal pathogens was seen in this study with *L. terebrantis* showing high variability in lesion and occlusion length among the seedling families tested as compared to the other three fungal species. Among the 23 loblolly pine families examined, the fungal species tested showed significant variability in

symptomatology expression. But within each family, all four fungal species had similar effects without significant lesion differences as indicated by non-significant interaction of the fungi and families. As far as seedling host response to wounding, two controls were used; a wound control and wound+media control. Neither of these treatments was detrimental to the seedlings and did not add to the lesion length. Wounding the stem of pine seedlings does not produce lesions; instead forms a callus tissue that encloses the wound (Eckhardt et al. 2004a; Klepzig et al. 1995; Matusick and Eckhardt 2010a). Therefore, creating a wound in the seedling stem does not add to mortality. Rather than forming resin soaked lesions, the wounded area in the control treatments was found to have a healed appearance with visible wound mark on the seedlings stem. Control wounds were found to have significantly smaller lesions and did not produce occluded tissue when compared to the fungal inoculations.

Dark brown, raised, and sunken lesions were observed on seedling stems following inoculations with fungi. In cross section, deformation of seedling stems was observed showing only small portions of living tissue remaining. Girdling of stems leading to mortality has been found in an earlier study following inoculations with *L. terebrantis* (Wingfield 1983). Lesions produced due to fungal inoculations extended beyond the wounded area to both sides on the stem of the seedlings. In certain cases the lesions failed to extend beyond the wounded area, especially in the seedlings inoculated with *L. procerum.* Lesions extended vertically in most of the cases with some evidence of radial movement. Occlusion of vascular tissues is considered one of many host responses to fungal infection and has been used as a measure of seedling response to inoculation with blue stain fungi (Nevill et al. 1995). Generally, occlusion lengths are similar to lesion length and further support the virulence testing of ophiostomatoid fungi (Eckhardt et al. 2004a). Occlusion of the xylem was observed (as unstained tissue) after removing the bark tissue where the occlusion was observed extending the entire lesion length. In many cases occlusion length did not extend the lesion length. Similar lesion and occlusion response was noticed in the two consecutive years in separate studies.

Symptom development after inoculation of seedlings was localized in the form of lesions and occlusions. A small fraction of the seedling died in the experiment (6.5%) during the year one. However, it is possible that mortality would be more prevalent if the experiment had continued for a longer time frame. Mortality associated with fungi was found in earlier experiments when the seedlings were kept for a longer period of time after imposing the inoculations (Harington and Cobb 1983; Wingfield 1983). Families L-14 and L-22 had less survival percentage as compare to other loblolly pine families tested in the first year.

The consistent re-isolation of *Leptographium* species from the inoculated seedlings indicated their ability to infect the pine seedlings. Similar results have been reported in a previous study but with lower re-isolations of fungal species from longleaf pine seedlings (Matusick et al. 2010). Mean root collar diameter (RCD) and height were not affected by the treatments though RCD and height were found to be significantly different among the loblolly pine families. This difference was attributed to the differences in RCD and height of the seedling stock used. Further, it indicates that despite the development of localized symptoms in the form of lesions, occlusions and tissue deformation, fungal infection did not produce whole tree symptomatology. The lack of symptomology is consistent with the previous trials conducted on southern pine seedlings (Matusick et al. 2010) and this was observed consistently. Significantly smaller final

root to shoot ratio among the seedlings inoculated with fungal treatments and wound control treatment indicated the decreased carbon allocation to the roots due to fungi. However, the root to shoot ratio could not be correlated to the lesion length overall.

Of all the seedling genotypes tested, families L-5, L-13, L-20 and L-8 had consistently smaller lesions overall and against the individual fungal species. Their host response is a good indication of being comparatively resistant to *Leptographium* species tested in these trials. Families L-1, L-2, L-3 and L-4 had consistently larger lesions in all cases and would indicate more susceptibility to these particular fungal species. Deployment of these families should take into account the relative risk of the site if there is some concern of pine decline. In the case of *L. procerum*, a known weak pathogen, failed to produce significant lesion lengths. However, the pine family ranking was found to behave like other fungal species included in the study. Similar to other inoculations trials that used slash pine, (Matusick et al. 2010), the 5 slash pine families tested were found to be generally more resistant to the fungi used than the loblolly pine families. Average lesion and occlusion length was less in slash pine families. A small set of five slash pine families included in the study showed less variability without differences in lesion length and occlusion length.

Generalized linear mixed model was fit to the data to test the breeding value of each family, conceptually similar to the mean family performance keeping the family effect random and treatments as fixed effect (Robinson 1991; Piepho et al. 2003). The general combining ability (GCA) estimates i.e. the best linear unbiased prediction (BLUP) estimates were used to rank the families on the basis of lesion length. When the families were kept random, interaction of family and fungal treatment was found to be significant, which is inconsistent to the results when the families and treatments were kept fixed. While ranking the families on the basis of lesion length overall, it was noticed that among the families L-20, L-13, L-8, L-18, L-11 and L-5 ranked lower while the families L-1, L-2, L-3 and L-4 ranked highest among the families tested. These results are consistent to the family grouping from the fixed model analysis further supporting the indication of some seedling families being comparatively resistant to *Leptographium* as compared to others. However, the family ranking was not consistent for each fungal treatment which is due to significant effect of fungal treatments.

Further studies should be done with some of the genotypes identified in the two year screening trials and focused on identifying and characterizing the source of resistance to *Leptographium.* Also, studying the correlations between seedling family performance and mature trees concerning *Leptographium* species and pine decline should be considered.

REFERENCES

Baker, J.B., and Langdon, O.G. 1990. *Pinus taeda* L., Loblolly Pine. *In* Silvics of North America, Vol. 1: Conifers. *Edited by* R.M. Burns, and B.H. Honkala. U.S. Dep. Agric. Agric. Handb. 654, Washington, D.C. pp. 497-512.

Barnard, E.L., Gilly, S.P., and Dixon, W.N. 1991. Incidence of *Heterobasidion annosum* and other root-infecting fungi in residual stumps and roots in thinned slash pine plantations in Florida. Plant Dis. 75: 823-828.

Bertagnole, C.L., Woo., J.Y., and Partridge, A.D. 1983. Pathogenicity of five *Verticicladiella* species to lodgepole pine. Can. J. Bot. 61: 1861-1867.

Brown, H.D., and McDowell, W.E. 1968. Status of loblolly pine die-off on the Oakmulgee District, Talladega National Forest, Alabama - 1968. U.S. Dep. Agric. For. Serv. Rept. No. 69-2-28.

Cobb, F.W. 1988. *Leptographium wageneri*, cause of black-stain root disease: a review of its discovery, occurrence and biology with emphasis on pinyon and ponderosa pine. *In Leptographium* root diseases on conifers. *Edited by* T.C. Harrington and F.W. Cobb. American Phytopathological Society, St. Paul, MN.

Dochinger, L.S. 1967. *Leptographium* root decline of eastern white pine. Phytopathology 57: 809.

Eckhardt, L.G., and Menard, R.D. 2009**.** Declining loblolly pine stands: symptoms, causes and management options. AL Treasured Forest Magazine Volume XXV111, No. 2, pp. 10-12.

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

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 Sci. 53(1): 84-92.

Harrington, T.C., and Cobb F.W. 1983. Pathogenicity of *Leptographium* and *Verticicladiella* spp. isolated from roots of western North American conifers. Phytopathology 73: 596-599.

Haynes, R. W., Adams, D. M., Alig, R. J., Ince, P. J., Mills, J. R., and Zhou, X. 2007. The 2005 RPA timber assessment update. Gen. Tech. Rep. PNW-GTR-699. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. pp. 212.

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. Proceedings of the eleventh biennial southern silvicultural research conference. Gen. Tech. Rep. SRS–48.: U.S. Department of Agriculture, Forest Service, Southern Research Station, Asheville, NC. pp. 622.

Klepzig, K.D., Raffa, K.F., and Smalley, E.B. 1991. Association of an insect-fungal complex with red pine decline in Wisconsin. Forest Sci. 37(4): 1119-1139.

Li, B., McKeand, S.E., and Allen, H.L. 1991. Genetic variation in nitrogen use efficiency of loblolly pine seedlings. Forest Sci. 37(2): 613-626.

Manion, P. D. (*Editor*). 1981. Tree disease concepts. Prentice-Hall, Inc. NJ.

Matusick, G., and Eckhardt, L.G. 2010a. Variation in virulence among four root-inhabiting ophiostomatoid fungi on *Pinus taeda* L., *P. palustris* Mill., and *P. elliottii* Englem. seedlings. Can. J. Plant Path. 32: 361-367.

Matusick, G., and Eckhardt, L.G. 2010b. The pathogenicity and virulence of four Ophiostomatoid fungi on young Longleaf pine trees. Can. J. Plant Path. 32(2): 172-176.

Matusick, G., Eckhardt, L.G., and Somers, G. 2010. The pathogenicity of four *Leptographium* species to mature longleaf pine roots. For. Ecol. Mgmt. 260: 2189-2195.

Nevill, R.J., Kelley, W.D., Hess, N.J., and Perry, T.J. 1995. Pathogenicity to loblolly pines of fungi recovered from trees attacked by southern pine beetles. South. J. Appl. For. 19(2): 78-83.

Otrosina, W. J., Hess, N. J., Zarnoch, S. J., Perry, T. J., and Jones, J. P. 1997. Blue-stain fungi associated with roots of southern pine trees attacked by the southern pine beetle, *Dendroctonus frontalis*. Plant Dis. 81: 942-945.

Piepho, H.P., Büchse, A., and Emrich K. 2003. A hitchhiker's guide to mixed models for randomized experiments. J. Agron. Crop Sci. 189: 310-322.

Robinson, G.K., 1991. That BLUP is a good thing: The estimation of random effects. Stat. Sci. 6: 15-51.

Shultz, R.P. 1997. Loblolly pine, the ecology and culture of loblolly pine (*Pinus taeda* L.). U.S. Dep. Agric., Forest Service, Agric. Handb. 713. Washington, D.C. pp. 1-16.

Smith, W.B., Patrik, D.M., Charles, H.P., and Scott, A.P. 2007. Forest Resources of the United States. A technical Document Supporting the Forest Service 2010 RPA Assessment.

Wear, D.N., and Greis, J.G. 2002. Southern forest resource assessment: summary of findings. J. Forest. 100: 6-14.

Wingfield, M.J. 1983. Association of *Verticicladiella procera* and *Leptographium terebrantis* with insects in the lake states. Can. J. For. Res. 13: 1238-1245.

Zanzot, J.W. 2009. Biology and ecology of root-feeding beetles and ophiostomatoid fungi in sandhills longleaf pine stands. Ph.D. Dissertation, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL.

Zanzot, J.W., Matusick, G., and Eckhardt, L.G. 2010. Ecology of root-feeding beetles and their associated fungi on longleaf pine in Georgia. Eviron. Entomol. 39(2): 415-423.

Zhou, X.D., De Beer, Z.W. Wingfield, B.D., and Wingfield, M.J. 2002. Infection sequence and pathogenicity of *Ophiostoma ips, Leptographium serpens* and *L. lundbergii* to pines in South Africa. Fungal Divers. 10: 229-240.

Figure 1. Loblolly pine seedling inoculated in the stem with *Leptographium.*

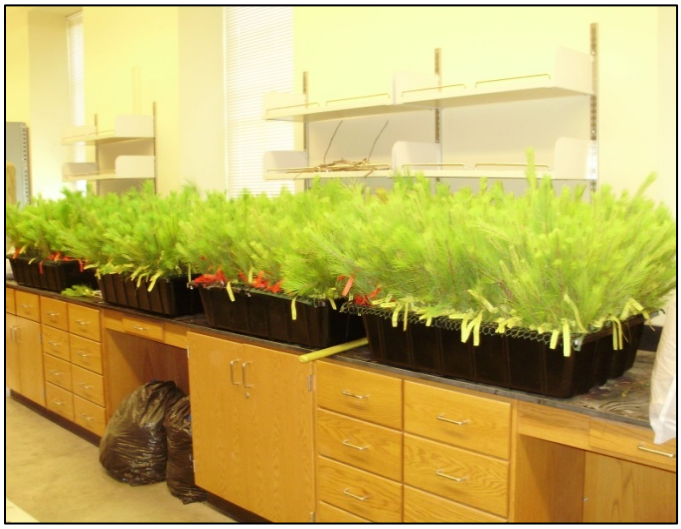

Figure 2. Seedlings placed in fast green solution for recording vascular tissue occlusion.

Figure 3. Brown lesions reported on seedlings stems following inoculations.

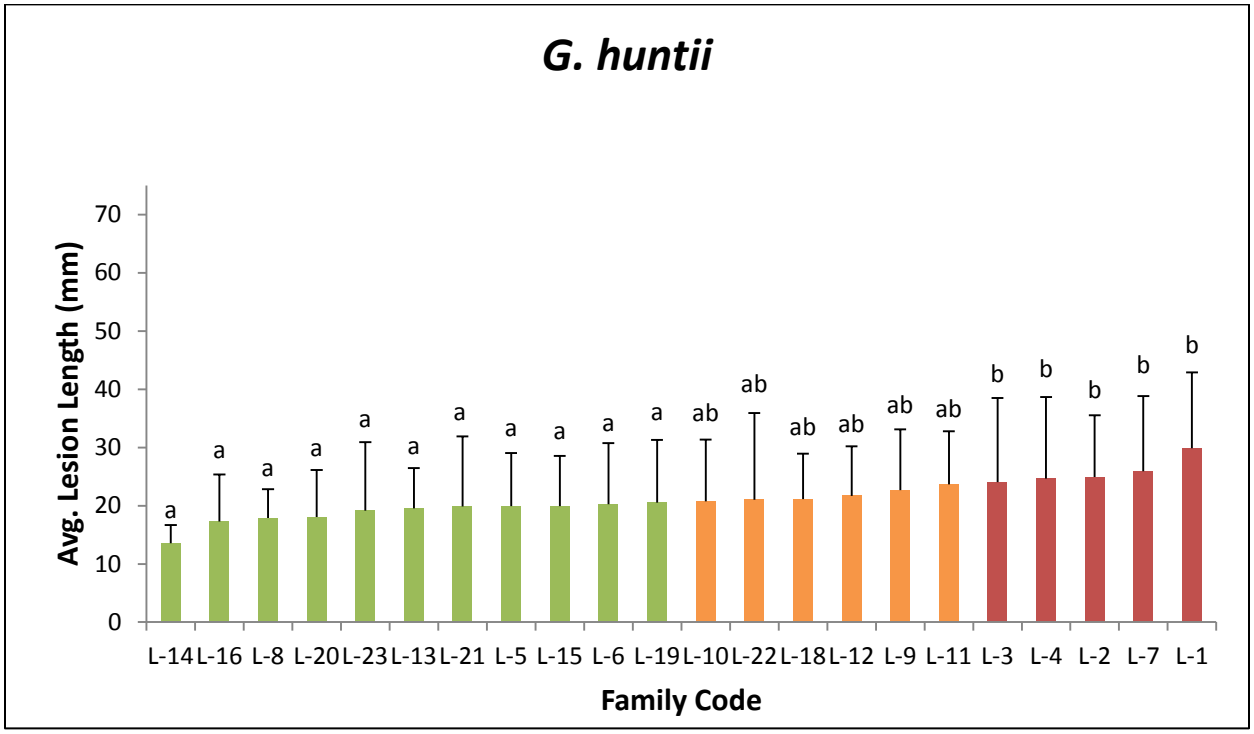

Figure 4. Average lesion length for loblolly pine families following inoculations with *Grosmannia huntii* from year one family screening.

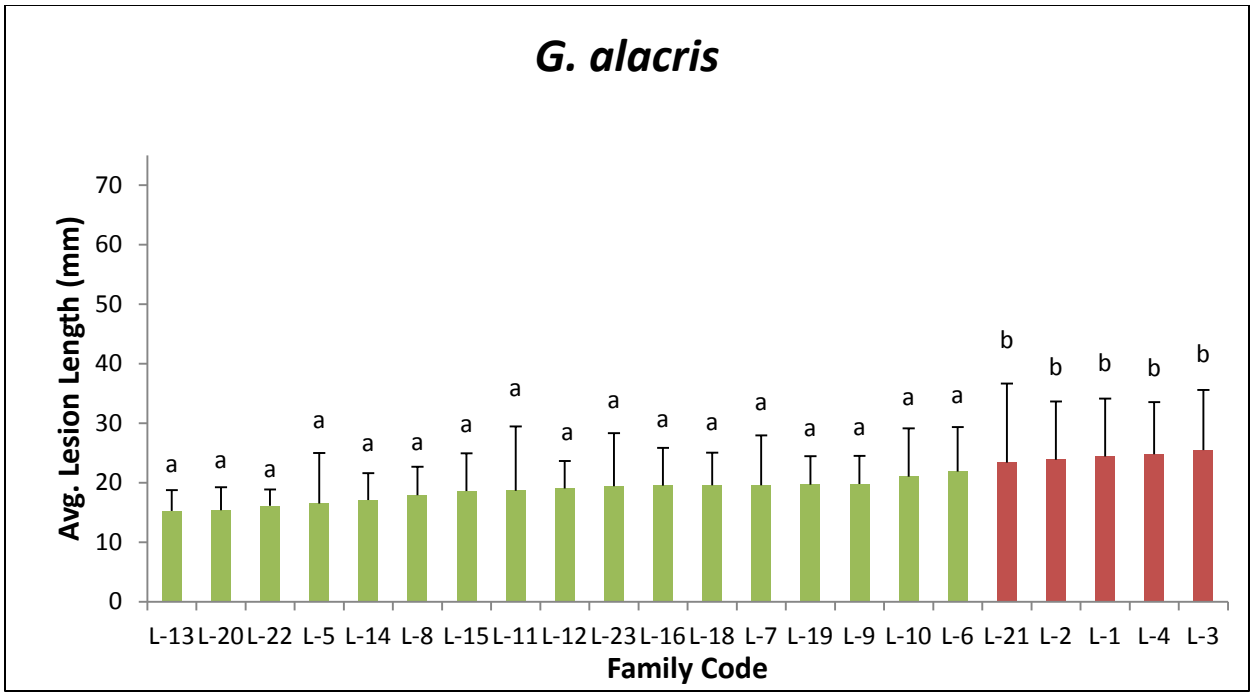

Figure 5. Average lesion length for loblolly pine families following inoculations with *Grosmannia alacris* from year one family screening.

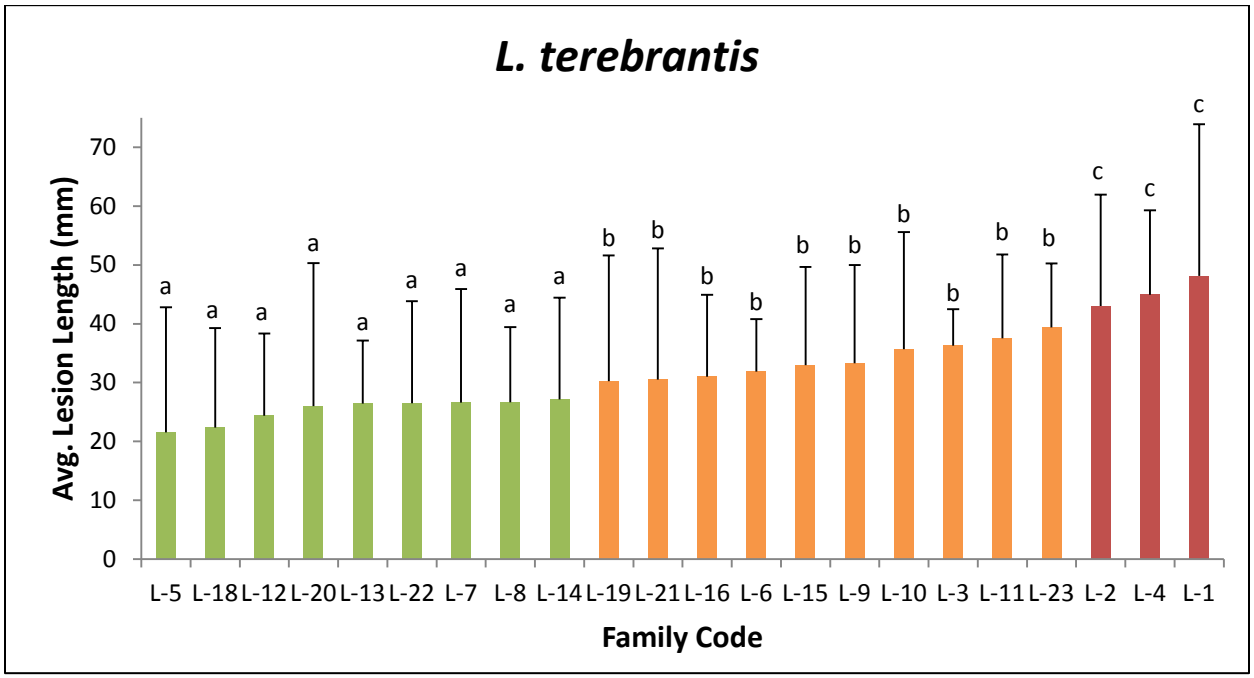

Figure 6. Average lesion length for loblolly pine families following inoculations with *Leptographium terebrantis* from year one family screening.

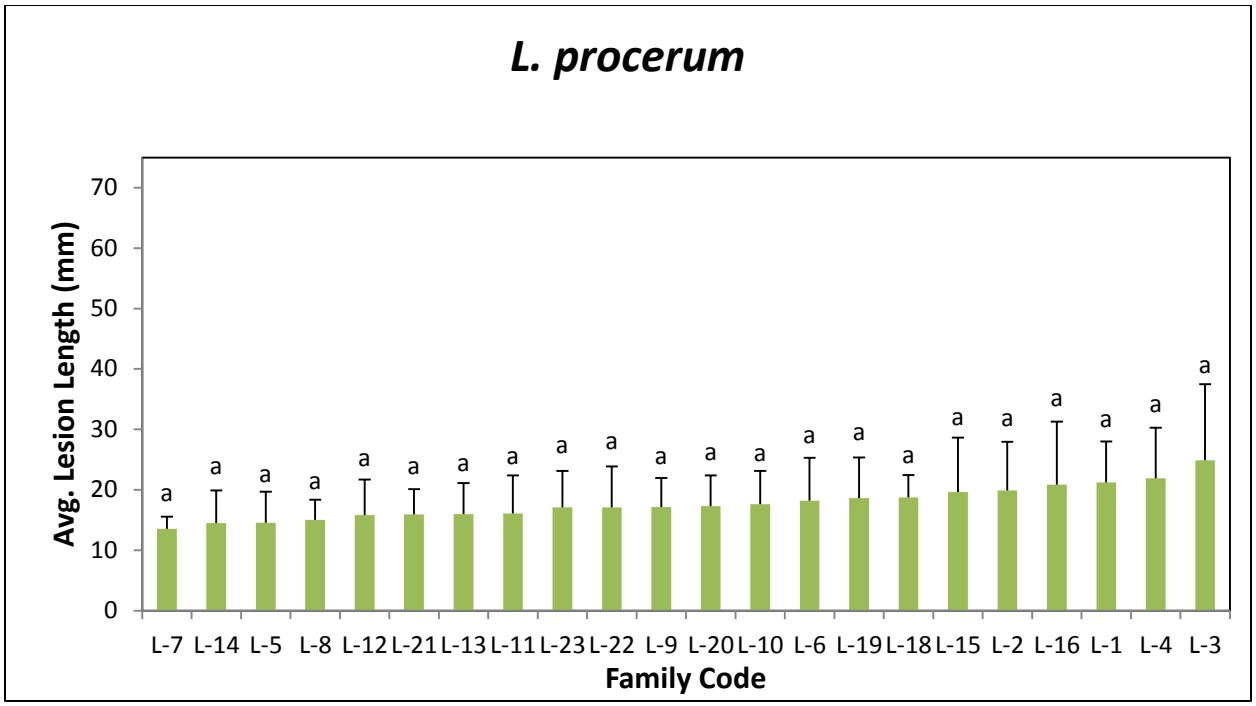

Figure 7. Average lesion length for loblolly pine families following inoculations with *Leptographium procerum* from year one family screening.

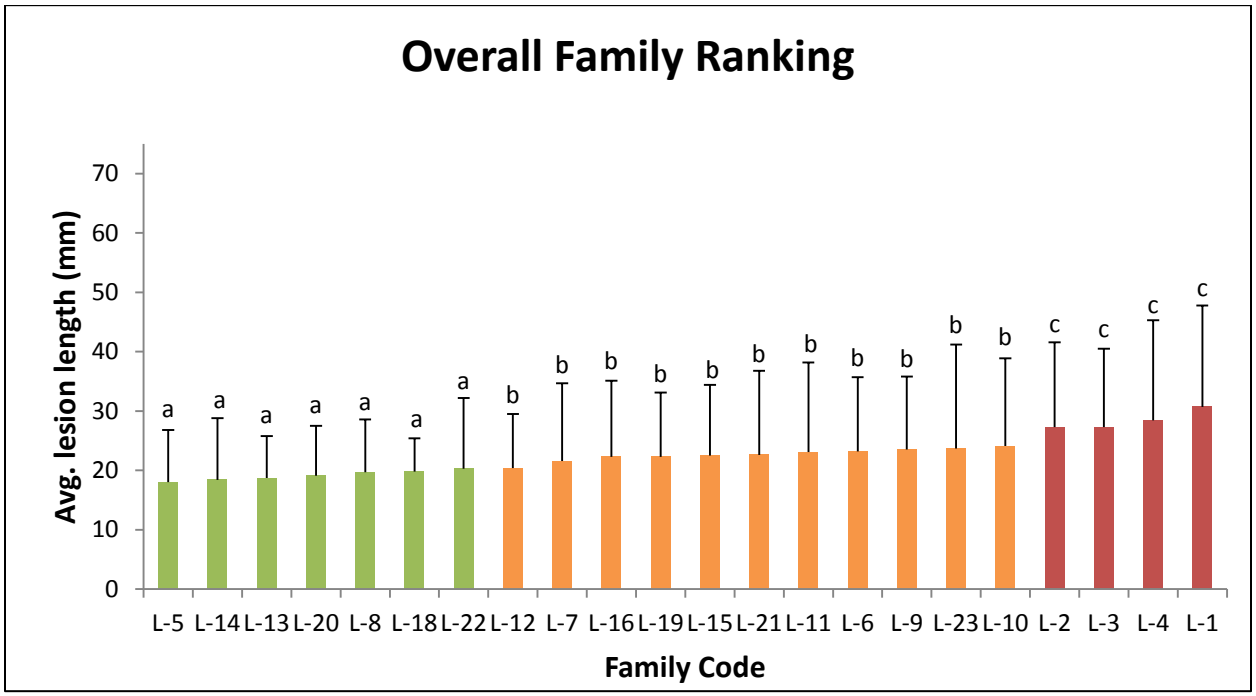

Figure 8. Average lesion length for loblolly pine families following inoculations with four ophiostomatoid fungal species from year one family screening.

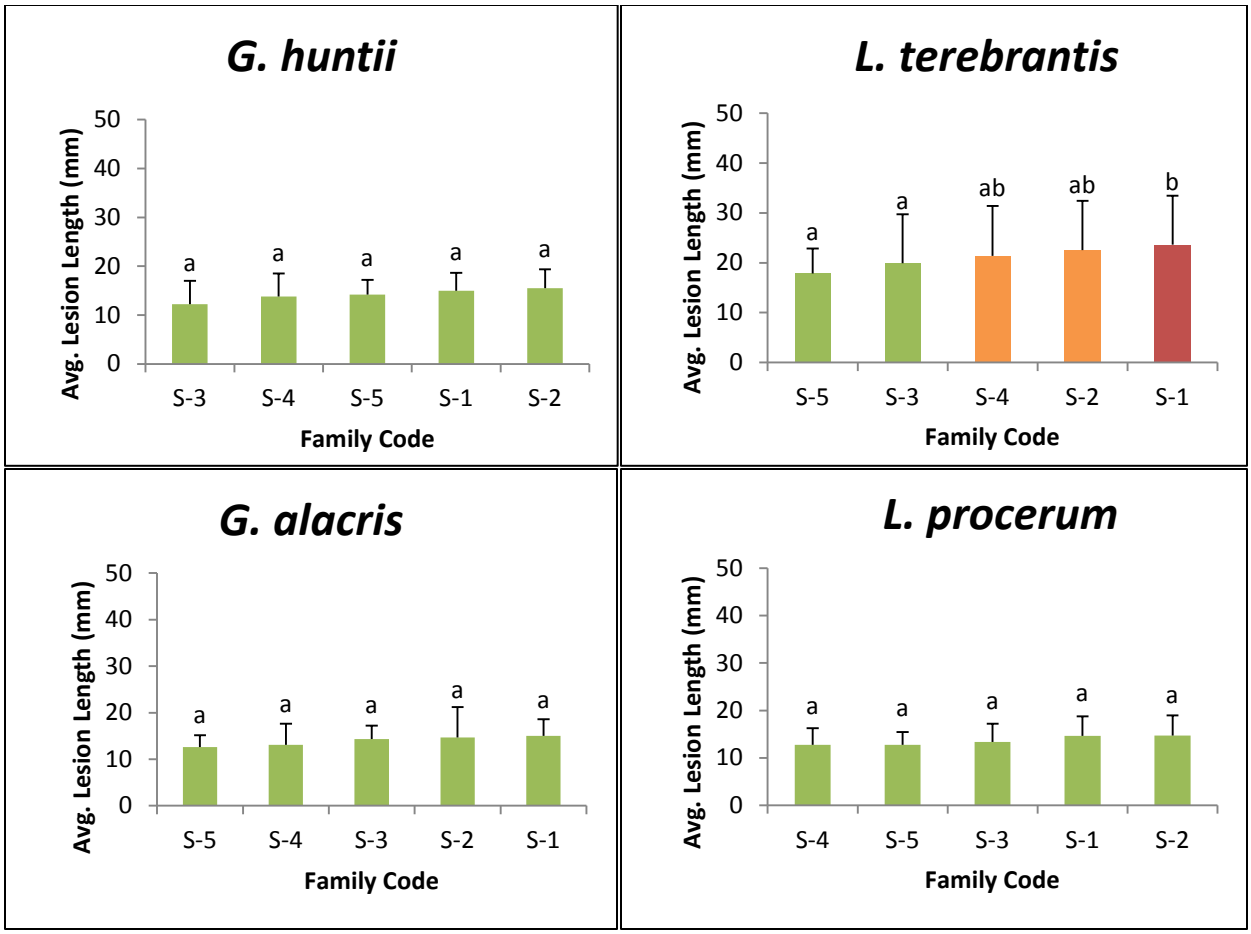

Figure 9. Average lesion length for slash pine families following inoculations with four ophiostomatoid fungal species from year one family screening.

Table 1. Pairwise comparison of treatments for lesion and occlusion length for loblolly pine families.

Treatment	Lesion Length	Occlusion Length
G. huntii* vs. *L. procerum	0.0073	0.0794
G. huntii* vs. *G. alacris	0.1277	0.1368
G. huntii* vs. *L. terebrantis	<0.0001	<0.0001
L. procerum* vs. *G. alacris	0.2349	0.7735
L. procerum* vs. *L. terebrantis	<0.0001	<0.0001
G. alacris* vs. *L. terebrantis	<0.0001	<0.0001
Wound vs. Wound-Media	0.8231	0.7986
Wound vs. *G. huntii*	<0.0001	<0.0001
Wound vs. *L. procerum*	<0.0001	<0.0001
Wound vs. *G. alacris*	<0.0001	<0.0001
Wound vs. *L. terebrantis*	<0.0001	<0.0001
Wound-Media vs. *G. huntii*	<0.0001	<0.0001
Wound-Media vs. *L. procerum*	<0.0001	<0.0001
Wound-Media vs. *G. alacris*	<0.0001	<0.0001
Wound-Media vs. *L. terebrantis*	<0.0001	<0.0001

Note: P- value <0.05 shows significant differences at alpha=0.05.

Table 2. Probability of greater F-value for lesion presence, occlusion presence, lesion length and occlusion length of loblolly pine families.

Effect	[1]DF	Lesion Presence	Occlusion Presence	Lesion Length	Occlusion Length	Lesion width	Lesion Depth
Block (B)	2	0.6096	0.6096	<0.0001	<0.0001	<0.0001	0.0159
Family (F)	21	0.7447	0.7447	<0.0001	0.0004	0.0020	0.1123
Trt (T)	3	0.6901	0.6901	<0.0001	<0.0001	<0.0001	<0.0001
TxF	63	0.7428	0.7428	0.9878	0.7744	0.7981	0.5950

[1]DF: degrees of freedom

Note: P- value <0.05 shows significant differences at alpha=0.05.

Table 3. Probability of greater F-value for final RCD and total height of loblolly pine families.

Effect	DF	Mean RCD	Mean Height
Block (B)	2	<0.0001	<0.0001
Family (F)	21	<0.0001	<0.0001
Trt (T)	5	0.9913	0.3328
TxF	105	0.2419	0.7119

Note: P- value <0.05 shows significant differences at alpha=0.05.

Table 4. Probability of greater F-value for lesion and occlusion length of slash pine families.

Effect	DF	Lesion	Occlusion	Lesion Length	Occlusion Length
Block (B)	2	0.1332	0.1335	0.0031	0.2419
Family (F)	4	0.3843	0.3802	0.2153	0.4478
Trt (T)	3	0.5402	0.5355	<0.0001	<0.0001
TxF	12	0.5191	0.5228	0.8039	0.8762

Note: P- value <0.05 shows significant differences at alpha=0.05.

Table 5. Average lesion length, occlusion length, seedling survival and fungal re-isolation frequency at the culmination of the study for loblolly pine families.

Family	Lesion Length (mm)	Occlusion Length (mm)	Survival (%)	Re-isolation (%)
L-5	18(8.8)	19.8(9.7)	90.9	90.8
L-14	18.4(10.4)	22.9(11)	80.0	98.6
L-13	18.7(7.1)	22.3(10.1)	91.9	93
L-20	19.1(8.4)	22.1(10.4)	98.7	85.1
L-8	19.7(8.9)	23.3(11.4)	94.7	90.6
L-18	19.8(5.6)	25.4(9.2)	91.3	92.9
L-22	20.3(11.9)	22.9(13.5)	86.2	94.6
L-12	20.4(9.1)	23.1(9.9)	93.1	91.7
L-7	21.6(13.1)	23.7(13.4)	93.3	97.8
L-16	22.3(12.8)	25.8(14.4)	92.6	90.9
L-19	22.3(10.8)	25.5(12.5)	99.4	89.5
L-15	22.5(11.9)	25.8(13.2)	99.2	86
L-21	22.6(14.2)	25.8(15.4)	97.4	87.9
L-11	23.1(15.1)	26.5(15.1)	90.9	84.1
L-6	23.2(12.5)	25.9(13.3)	100	90.3
L-9	23.5(12.3)	27.4(14.5)	97.5	90
L-23	23.7(17.5)	26.8(18)	94.2	91.9
L-10	24.1(14.8)	28.7(16.8)	92.9	96.2
L-2	27.3(14.3)	30.8(14.5)	97.9	85.7
L-3	27.3(13.2)	30.5(14.3)	92.8	80
L-4	28.4(16.9)	31.6(17.3)	96.7	90.6
L-1	30.7(17.1)	33.8(17.7)	98.7	83.2

Note: Means and standard deviations (parentheses).

Table 6. Average lesion length, occlusion length, seedling survival and fungal re-isolation frequency at the culmination of the study for slash pine families.

Family	Lesion Length (mm)	Occlusion Length (mm)	Survival (%)	Re-isolation (%)
S-5	14.4(4.2)	16.6(6.5)	89.7	87.3
S-3	14.8(6)	17.9(8.2)	85.3	89.1
S-4	15(6.7	16.8(8.5)	87.3	92.2
S-2	17(7.5)	20.6(9.9)	85.5	89.2
S-1	17.4(7.4)	19.8(9.2)	92.2	88.9

Note: Means and standard deviations (parentheses).

Table 7. Pairwise comparison of treatments for lesion and occlusion length for loblolly pine families (mixed model).

Treatment	Lesion Length	Occlusion Length
G. huntii* vs. *L. procerum	<0.0001	0.0066
G. huntii* vs. *G. alacris	0.0517	0.0875
G. huntii* vs. *L. terebrantis	<0.0001	<0.0001
L. procerum* vs. *G. alacris	0.0407	0.2999
L. procerum* vs. *L. terebrantis	<0.0001	<0.0001
G. alacris* vs. *L. terebrantis	<0.0001	<0.0001
Wound vs. Wound-Media	<0.7990	<0.7864
Wound vs. *G. huntii*	<0.0001	<0.0001
Wound vs. *L. procerum*	<0.0001	<0.0001
Wound vs. *G. alacris*	<0.0001	<0.0001
Wound vs. *L. terebrantis*	<0.0001	<0.0001
Wound-Media vs. *G. huntii*	<0.0001	<0.0001
Wound-Media vs. *L. procerum*	<0.0001	<0.0001
Wound-Media vs. *G. alacris*	<0.0001	<0.0001
Wound-Media vs. *L. terebrantis*	<0.0001	<0.0001

Note: P- value <0.05 shows significant differences at alpha=0.05.

Table 8. Covariance parameter estimates are presented. Variation among families is significantly different from zero for lesion length, and occlusion length. Family by treatment interaction is also significant; families can be ranked overall and for each fungus individually.

Cov Parm	Lesion Length	Lesion Width	Lesion Depth	Occlusion Length
Family (F)	0.0261	0.1219	0.0514	0.0352
TxF	0.0011	0.1729		0.0009
BxF	0.0022	0.0130	0.1424	0.0050
Residual	<0.0001	<0.0001	<0.0001	<0.0001

Note: P- value <0.05 shows significant differences at alpha=0.05.

Table 9. Type 3 tests of fixed effects (F tests) suggest that all the effects are significant for lesion length, lesion width, lesion depth and occlusion length.

Effect	DF	Lesion Length	Lesion Width	Lesion Depth	Occlusion Length
Block (B)	2	<0.0001	<0.0001	<0.0001	<0.0001
Trt (T)	1	<0.0001	<0.0001	<0.0001	<0.0001
BxT	15	<0.0001	<0.0001	0.0008	<0.0001

Note: P- value <0.05 shows significant differences at alpha=0.05.

Table 10. Families ranking for lesion length (estimate) overall and across all the four treatments.

Family	Estimate	Overall Rank	Estimate	Rank GH	Estimate	Rank LT	Estimate	Rank GA	Estimate	Rank LP
L-20	-2.934	1	0.0223	14	-2.867	2	-0.628	4	1.6457	22
L-13	-2.215	2	0.6403	17	-1.415	6	-0.846	2	0.2414	13
L-8	-1.981	3	-0.342	9	-0.8	11	0.1202	14	-0.212	10
L-18	-1.865	4	-0.477	7	-1.714	4	0.397	18	0.6327	15
L-11	-1.547	5	0.36	15	-0.386	14	0.0744	13	-1.012	5
L-5	-1.376	6	1.4366	22	-3.462	1	0.2601	16	0.9082	19
L-16	-1.281	7	-0.481	6	-1.534	5	-0.189	10	1.4068	21
L-14	-1.046	8	-0.682	5	0.0474	15	0.1405	15	-0.157	11
L-19	-0.812	9	-0.227	10	-1.397	7	0.344	17	0.7741	17
L-6	-0.621	10	-0.895	3	-1.841	3	1.387	21	0.9623	20
L-15	-0.421	11	-0.791	4	0.2479	16	-0.508	5	0.7892	18
L-7	-0.338	12	1.3217	21	-0.868	9	-0.409	7	-0.255	8
L-10	-0.334	13	0.0066	13	-0.615	12	0.7361	20	-0.335	7
L-9	-0.013	14	1.1918	20	-0.816	10	-0.137	11	-0.246	9
L-21	0.1309	15	-1.009	2	0.3034	17	1.8111	22	-1.024	4
L-12	0.21	16	0.9363	18	-1.067	8	-0.127	12	0.3889	14
L-22	0.3104	17	0.9648	19	-0.461	13	-0.419	6	0.1085	12
L-23	0.4748	18	-1.759	1	3.3046	20	-0.28	9	-0.97	6
L-2	3.1606	19	-0.034	12	2.5937	19	0.4372	19	-1.029	3
L-3	3.6794	20	-0.447	8	2.3807	18	-0.331	8	0.6889	16
L-4	4.2569	21	-0.204	11	4.9687	21	-0.772	3	-1.342	2
L-1	4.5598	22	0.4685	16	5.3972	22	-1.06	1	-1.967	1

GH - *Grosmannia huntii*, LT - *Leptographium terebrantis*, GA – *Grosmannia alacris*, LP - *Leptographium procerum*