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Intraspecies variation of mature *Pinus taeda* in response to root-infecting ophiostomatoid fungi

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Summary

Seedling screening studies have shown intraspecies variation in susceptibility of *Pinus taeda* (loblolly pine) to *Leptographium terebrantis* and *Grosmannia huntii*, the causal agents of root infection in *Pinus* species. However, it is critical to understand the susceptibility of mature *P. taeda* trees. 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 vascular occlusion were recorded 8 weeks later. Families previously considered as susceptible had relatively longer lesions and occlusions. The variation in susceptibility to the two fungi remained the same as exhibited by families in the seedling trial. These results suggest intraspecies variation in relative susceptibility of *P. taeda* to *L. terebrantis* and *G. huntii* remains similar regardless of the age of the tree.

1 | INTRODUCTION

Pinus taeda L., an important timber species, is commercially planted on approximately 11.73 million hectares of land in the southern United States (Baker & Langdon, 1990; Rauscher, 2004). This species, when fertilized, can produce high wood volume per hectare yields (Fox, Jokela, & Allen, 2004) providing all-purpose marketable forest products such as furniture, pulpwood, composite boards, crates, boxes, and pallets (Schultz, 1997). A factor impacting on the growth and optimal productivity of this important pine species is root-infecting pathogenic fungi, among which some are associated with pine decline (Eckhardt, Jones, & Klepzig, 2004; Otrosina, Bannwart, & Roncadori, 1999).

Pine decline (PD), a decline disease syndrome, was first observed by Brown and McDowell (1968) on the Talladega National Forest in the Oakmulgee Ranger District located in central Alabama, USA. Brown and McDowell (1968) reported 40- to 50-year-old *P. taeda* stands with symptoms of decline that included thinning crowns, reduced radial growth, and root deterioration. This decline syndrome has subsequently been reported across the south-eastern United States (Eckhardt, Weber, Menard, Jones, & Hess, 2007; Hess et al., 2002).

The decline disease spiral, the widely accepted model of forest decline, given by Manion (1981) involves interaction of three 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 playing a role at the end (contributing factors). In the context of PD, tree genetics and increased slopes are the predisposing factors, drought and ozone are the inciting factors, and root-feeding bark beetles and their associated ophiostomatoid fungi are the contributing factors (Eckhardt, Goyer, Klepzig, & Jones, 2004; Eckhardt, Jones et al., 2004; Eckhardt & Menard, 2008; Eckhardt et al., 2007). 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 & 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, Goyer et al., 2004; Eckhardt, Jones et al., 2004). *Leptographium terebrantis* and *G. huntii* were found to be more pathogenic to *Pinus* spp. than other ophiostomatoid fungi (Matusick, Eckhardt, & Somers, 2010; Singh, Anderson, & Eckhardt, 2014). The knowledge of the interspecies and intraspecies susceptibility of the tree host (a major predisposing factor) to these fungi is, however, limited in the literature.

Ophiostomatoid fungi cause lesions in the phloem and occlusion in the xylem of artificially inoculated stems of seedlings (Eckhardt, Jones et al., 2004) and stems (Matusick, Nadel, Walker, Hossain, & Eckhardt, 2016) and roots (Matusick et al., 2010) of mature *P. taeda*

trees. Furthermore, these fungi use sap sugars, defence compounds and sugars leaking from degraded cell walls to survive 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 & Thomma, 2013). Fungal spread and tyloses formation both, however, disturb plant water transport (Joseph, Kelsey, & Thies, 1998). Moreover, investment of the tree in defence can occur at the expense of radial growth (Krokene, Nagy, & Solheim, 2008).

Artificial inoculation of *P. taeda*, *Pinus palustris* Mill. (longleaf pine) and *Pinus elliotti* Englem. (slash pine) by Matusick et al. (2010) with *Grosmannia* and *Leptographium* species showed interspecies variation in susceptibility/tolerance to the inoculated fungi with *P. taeda* being the most susceptible among three *Pinus* species. Furthermore, Singh et al. (2014) conducted a seedling screening study to examine intraspecies variability in tolerance of *P. taeda* to *G. huntii* and *L. terebrantis*. Results showed that *P. taeda* families have varying levels of susceptibility/tolerance to those fungi. The reliability of results obtained from families at the seedling stage is yet to be answered as the susceptibility may vary by age of the trees. Thus, the aim of this study was to examine the intraspecies 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 intraspecies variation in tolerance/susceptibility of *P. taeda* to ophiostomatoid fungi is an inherent character of a family independent of the age of the tree.

2 | MATERIALS AND METHOD

2.1 | Experimental design

Pinus taeda stands from four families in Alabama and Georgia (~31°53′N and 85°8′W, 81.16 m above the sea level) were chosen. Among four 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 relatively less tolerant (more susceptible) families as given in Table 1. 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 years old (for 2015) and 18 years 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.

2.2 | 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°C in malt extract agar (MEA) in the Forest Health Dynamics Laboratory at Auburn University. These fungal isolates were cultured on MEA 2 weeks before root inoculations. The *L. terebrantis* and *G. huntii* isolates used in the study were, respectively, isolated from the roots of *P. taeda* in the Talladega National Forest, Oakmulgee Ranger District, AL, USA, and the root of *P. palustris* in the Fort Benning Military Reservation, GA, USA, exhibiting symptoms of decline such as localized tissue damage and defoliating crown as described by Eckhardt et al. (2007). The *L. terebrantis* isolate used in the study was determined to be the most virulent isolate among 41 isolates maintained at the Forest Health Dynamics Laboratory (Devkota, 2017). Several previous artificial stem and root inoculation studies have used these isolates (Chieppa, Eckhardt, & Chappelka, 2017; Matusick et al., 2010; Singh et al., 2014).

TABLE 1 Overall lesion and occlusion length produced by *Grosmannia huntii* and *Leptographium terebrantis* in seedling screening study (Devkota, 2017; Singh et al., 2014)

Family	Lesion length (mm)	Occlusion length (mm)
T1	20 (8.9)	23 (11.4)
T2	18 (8.8)	20 (9.7)
S1	64 (41.5)	71 (43.3)
S2	34 (14.3)	73 (47.2)

Means followed by the standard deviation in parenthesis.

In the field, two primary lateral roots were excavated from each *P. taeda* tree without damaging roots with hand tools that had been sterilized using 70% ethanol. On each excavated root, two wounds at a depth to the cambium layer were created by gently hitting a rubber mallet on a 13-mm-diameter sterile steel arch punch. The two wounds were kept 30 cm apart as the length of the lateral root was limited. The root inoculation was then completed by removing the bark plug and placing the 10-mm agar plug (fungus-side-down) with actively growing fungi in the wound. The fungal treatments *L. terebrantis* and *G. huntii* and the control treatments wound with sterile media and wound without media were applied to each tree. 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 was sealed with duct tape to minimize any further contamination. The inoculation points of the roots were marked with labelled pin flags and covered with soil.

2.3 | Laboratory measurements

The inoculated roots were re-excavated and removed from the tree after 8 weeks. To prevent moisture loss, the exposed ends of the roots were painted with Drylok Latex Masonry Waterproofer (Scranton, PA, USA) and the root samples were transported to the laboratory at Auburn University for further processing. The diameter of each root at the point of inoculation was measured. To observe the necrotized and occluded tissues, bark around the inoculation area was scrapped off and painted with a solution of Fast Green stain (Fast Green FCF; Sigma Chemical Co.) (0.25 g/L of water). The length and width of the necrotic tissue were traced on a clear transparent sheet, and lesion area was determined using a Lasico® Planimeter (Lasico®, Los Angeles, CA, USA) as described by Matusick, Somers and Eckhardt (2012). The

length 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 discoloured sapwood was measured as occlusion depth. In the spring 2016 experiment, for a few samples, the length of the occlusion caused by the fungi exceeded 30 cm. The occlusion caused by the fungi compared to the overlapping control was easily distinguishable as it was found to occur deeper into the tissue and tapering towards the endpoint compared to that of the superficial occlusion present for the controls. To further confirm whether the occlusion was caused by either the fungus or control, occluded wood sections at multiple points were cut out and plated onto MEA amended with cycloheximide and streptomycin. Controls were removed from the analyses when the inoculated fungus was reisolated from overlapping occluded tissue for which we could not distinguish between the fungus and that of the control. Small pieces of stem tissue from the distal and proximal portions of the inoculation site of every root were plated to reisolate the inoculated fungi.

2.4 | Statistical analysis

Data were analysed using the 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 included in the model. Root diameter was used as the 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 all the response variables such as lesion and occlusion significantly differed by study period (replication), data were analysed separately for each inoculation period.

The general linear model used can be expressed as follows:

$$y_{ik} = \beta_0 + T_i + F_k + TF_{ik} + \text{coV} + \mathcal{E}_{ik},$$

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

3 | RESULTS

During both summer 2015 and spring 2016, the inoculation of *L. terebrantis* and *G. huntii* into the roots of mature *P. taeda* resulted in necrosis around the inoculation points. In addition, resin soaking at the cross section occurred above and below the initial point of inoculation. The two control inoculations resulted in significantly smaller lesions than that caused by fungal inoculation in all the tested families. So, only the effects of the fungal inoculation were analyzed further. The families responded differently to the fungal inoculation after two different inoculation periods in terms of lesion length ($F_{3,374} = 7.69$, $p \leq .0001$; Figure 1), lesion area ($F_{3,374} = 10.48$, $p \leq .0001$; Figure 2) and occlusion length ($F_{3,374} = 3.68$, $p = .01$; Figure 3). The lesion area and length, and occlusion length and width were more severe in the spring compared to that of the summer (Figures 2 and 3). Hence, the data from summer and spring root inoculation studies were analyzed separately.

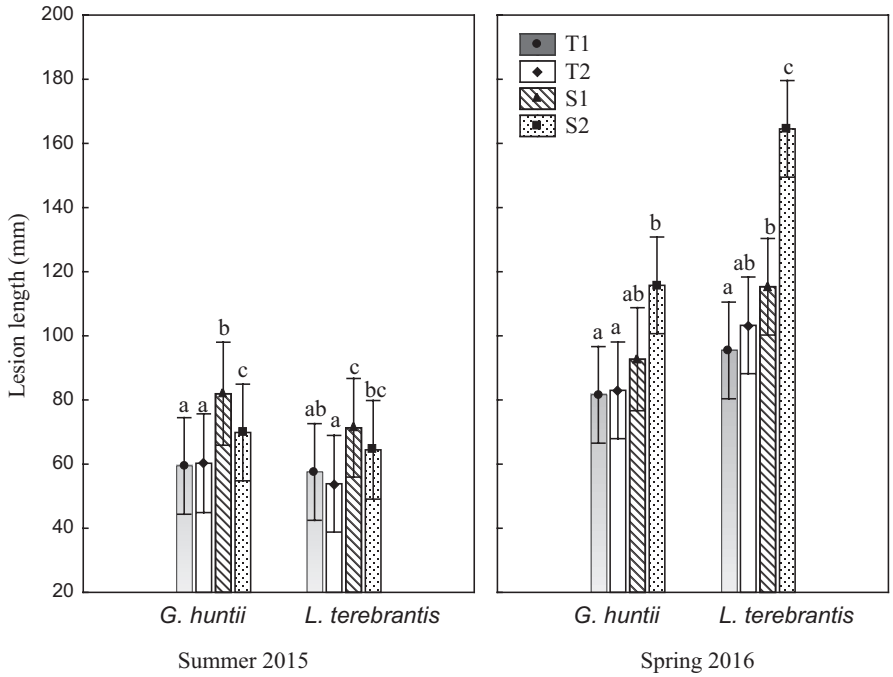

FIGURE 1 Lesion length caused by the two different fungi in summer 2015 and spring 2016 inoculations. T1 and T2: families tolerant to root-infecting ophiostomatoid fungi at seedling stage. S1 and S2: families susceptible to root-infecting fungi at seedling stage. Error bars indicate 95% confidence intervals. Different letters represent significant differences among families within each fungus at $\alpha = .05$

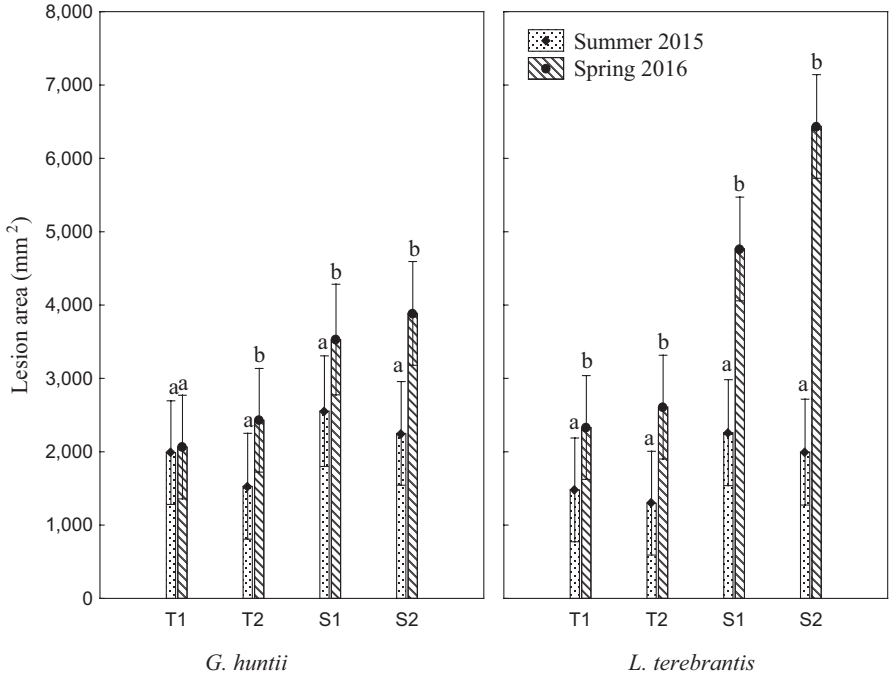

FIGURE 2 Lesion area caused by two different fungi in summer 2015 and spring 2016 inoculations. T1 and T2: families tolerant to root-infecting ophiostomatoid fungi at seedling stage. S1 and S2: families susceptible to root-infecting ophiostomatoid fungi at seedling stage. Error bars indicate 95% confidence intervals. Different letters indicate the significant differences lesion area between two inoculation periods within each family at α = .05

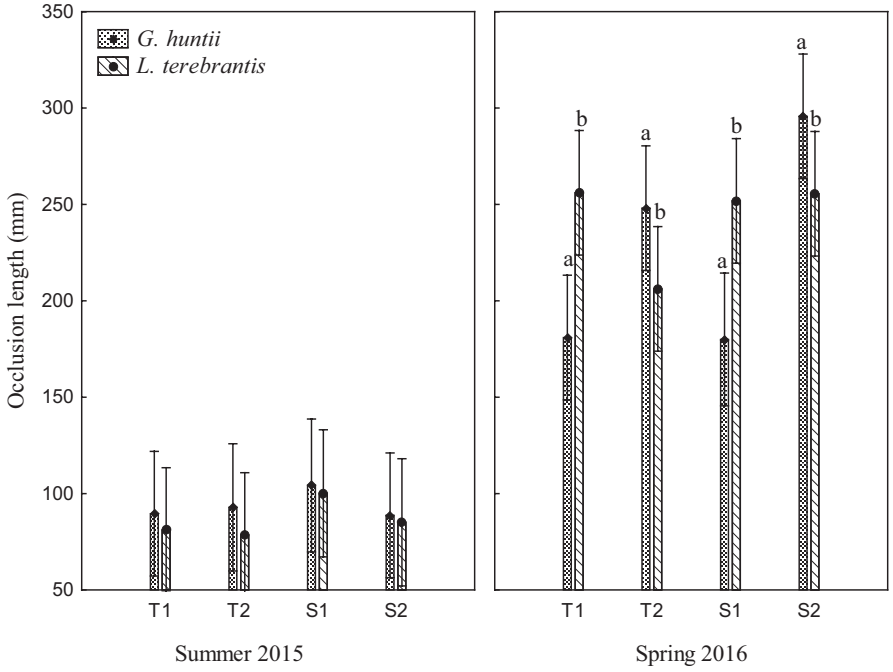

FIGURE 3 The occlusion length caused by the two different fungi in summer 2015 and spring 2016 inoculations. T1 and T2: families tolerant to root-infecting ophiostomatoid fungi at seedling stage. S1 and S2: families susceptible to root-infecting ophiostomatoid fungi at seedling stage. Error bars indicate 95% confidence intervals. Different letters indicate the significant differences in the occlusion length within each family at α = .05

3.1 | Summer 2015 inoculation

Families differed in their overall response to the fungal inoculation during summer 2015 ($F_{21,543}$ = 3.01, $p \leq .0001$) (Table 2). However, within a single family, the pathogenicity of the two fungi was not different ($F_{21,543}$ = 1.07, p = .38). *Leptographium terebrantis* and *G. huntii* did not differ in their virulence (in terms of occlusion length; $F_{7,179}$ = 1.41, p = .21; Figure 3).

Four *P. taeda* families differed in their response to inoculated fungi in terms of the area ($p \leq .0001$) and length of the lesion ($p \leq .0001$), and length (p = .03) and depth ($p \leq .0001$) of the vascular occlusion (Table 2). The lesion area did not differ significantly between two families (T1 and T2) tolerant to ophiostomatoid fungi (p = .07) and also between two families (S1 and S2) susceptible to ophiostomatoid fungi (p = .30) (Table 3). The lesion area observed in tolerant family T2 was substantially smaller than susceptible families S1 and S2 (Table 3). Susceptible family S1 had the highest 2,400.76 ± 1,553.76 mm^2, and tolerant family T1 had the lowest 1,734.44 ± 1,010.62 mm^2 mean lesion area among four families. Families showed a similar trend for lesion length. The mean lesion length for families S1, S2, T1, and T2 were

TABLE 2 *p*-values for family and fungi main effects 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	.01	.07	.01	.08	.65	<.0001	.02
	Fam	3	<.0001	<.0001	.31	.03	.12	.10	<.0001
	Trt	1	.08	.06	.10	.10	.58	.34	.02
	Fam*trt	3	.85	.77	.46	.86	.38	.94	.51
2016	RD	1	.03	.86	<.0001	.99	<.0001	<.0001	<.0001
	Fam	3	<.0001	<.0001	<.0001	.03	.02	.43	.12
	Trt	1	.002	<.0001	.62	.31	.31	.10	<.0001
	Fam*Trt	3	.03	.32	.54	.01	.37	.73	.29

RD, root diameter; Fam, family; Trt, fungal treatment.
p-values were estimated at $\alpha = .05$.

76.41 ± 28.38, 67.23 ± 22.66, 58.50 ± 18.62, and 57.02 ± 12.46 mm respectively. Susceptible family (S1) had a significantly longer occlusion length compared to tolerant families (T1 and T2) (Table 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 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.

3.2 | Spring 2016 inoculation

Pinus taeda families responded differently to the fungal inoculation in spring 2016 ($F_{21,552} = 4.63$, $p \leq .0001$) (Figure 1 and Table 2). However, family and fungal treatment interaction were significant ($F_{21,552} = 2.27$, $p \leq .0001$), 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 ($p = .03$) and occlusion length ($p = .01$) (Table 2). *Leptographium terebrantis* caused significantly longer occlusion length than *G. huntii* in all of the families (Figure 3).

Area of the lesions caused by both fungi was significantly smaller in the roots of tolerant families (T1 and T2) compared to the susceptible families (S1 and S2) (Table 4). *Leptographium terebrantis* and *G. huntii* respectively caused the largest mean lesion area of 6,435.40 ± 4,610.77, and 3,886.36 ± 2,524.02 mm^2 in susceptible family S2 compared to the other families. *Leptographium terebrantis* caused mean lesion length of 115.31 ± 41.78, 164.52 ± 96.08, 95.44 ± 31.40, and 103.26 ± 26.31 mm in families S1, S2, T1, and T2 respectively. Similarly, *G. huntii* caused mean lesion length of 92.71 ± 37.39, 115.75 ± 62.44, 81.59 ± 21.57, and 83.01 ± 34.94 mm in families S1, S2, T1, and T2 respectively. 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 four *P. taeda* families (Table 4). Both fungi caused lesions with a larger area, and longer and wider length in susceptible family S2 followed by susceptible S1 (Table 4).

The success of re-isolation of *L. terebrantis* from inoculated root 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. Consistency in re-isolation of the same fungi which was used for the inoculation proved the success of fungal inoculation and infection.

4 | DISCUSSION

This is the first study to show within-species variation in susceptibility of mature *P. taeda* to ophiostomatoid fungi, largely confirming previous findings from the seedling screening study conducted by Singh et al. (2014) and Devkota (2017). Mature trees from the susceptible families S1 and S2 and tolerant families T1 and T2 showed similar

TABLE 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 (230.30)	.07	2.67 (4.27)	.53	1.49 (6.10)	.80	−0.08 (0.58)	.89
T1 vs S1	−545.09 (235.31)	.02	−16.41 (4.37)	.0002	−14.63 (6.16)	.01	1.54 (0.59)	.01
T1 vs S2	−305.25 (229.76)	.18	−7.67 (4.26)	.07	−0.13 (6.01)	.98	1.59 (0.58)	.007
T2 vs S1	−964.87 (232.88)	<.0001	−19.09 (4.32)	<.0001	−16.12 (5.99)	.008	1.62 (0.57)	.006
T2 vs S2	−725.02 (229.04)	.002	−10.34 (4.25)	.02	−1.62 (6.10)	.78	1.66 (0.58)	.004
S1 vs S2	239.84 (233.05)	.30	8.74 (4.33)	.04	−14.50 (6.09)	.01	0.04 (0.58)	.94

T1 and T2: families tolerant to root-infecting ophiostomatoid fungi at the seedling stage and S1 and S2: families susceptible to root-infecting ophiostomatoid fungi at the seedling stage. Estimates followed by standard errors in parenthesis. *p*-values show significant differences at $\alpha = .05$.

patterns of tolerance to root-infecting ophiostomatoid fungi as shown at the seedling stage. Intraspecific variation in susceptibility of mature *Pinus* species to ophiostomatoid fungi has been observed in some other conifer hosts (Rice, Thormann, & Langor, 2007a,b). Several other researchers have reported considerable variation in susceptibility to pitch canker within *P. taeda* (Dwinell & Phelps, 1977; Gordon, Okamoto, Storer, & Wood, 1998; Kelley & Williams, 1982; Schultz, Gordon, & McCain, 1990) and *P. radiata* (Correll et al., 1991; Roux et al., 2007). The present study shows that there is great potential for selecting *P. taeda* families tolerant to ophiostomatoid fungi from the current southern United States planting stock and thus ensuring potential losses due to these fungi can be minimized in the future.

The reliability of the use of seedlings to screen tolerance/susceptibility of *P. taeda* families to *L. terebrantis* and *G. huntii* was a question (Coyle et al., 2015). However, the results of our study confirmed the use of seedlings in screening studies is consistent and reliable. Certainly, differences in the actual lesion size to define family susceptibility levels are different between the present study when compared to that of Singh et al. (2014) as seedling size is proportionally a lot smaller than that of the mature root, but 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.

For the susceptible tree families, the *L. terebrantis* isolate used in the study was relatively more virulent in the spring, in terms of lesion length and area, compared to that of the *G. huntii* isolate. There was no variation in the virulence between the two fungal isolates in tolerant tree families, thus suggesting that the isolates may have varying potential to cause infection in susceptible tree families. Comparing the response of the same two fungal isolates used in this study, when inoculated into seedlings, found larger lesions for *L. terebrantis* compared to *G. huntii* (Singh et al., 2014). Matusick et al. (2016), however, inoculated the same fungal isolates into *P. taeda* roots in spring and found *G. huntii* to be relatively more virulent than that of *L. terebrantis*. These discrepancies may be attributed to the consideration of intraspecies *P. taeda* family susceptibility in our study which was not considered in the previous study. The virulence of the ophiostomatoid fungi may vary within a species (Devkota, 2017; Lieutier, Yart, Ye, Sauvard, & Gallois, 2004; Parmeter, Slaughter, Chen, Wood, & Stubbs, 1989). The present study utilized the most virulent *L. terebrantis* isolate among 41 isolates maintained by the FHDL. It is suggested that intraspecific variation in virulence of various fungal isolates should be considered when screening host species for disease tolerance.

Lesions and occlusions were observed in all of the roots of the inoculated trees which support the idea that the hypersensitive response occurs in *P. taeda* following fungal inoculation (Matusick, Eckhardt, & Enebak, 2008; Matusick et al., 2010, 2012; Otrosina, Walkinshaw, Zarnoch, Sung, & Sullivan, 2000). The response observed in the wound and wound with sterile media-treated roots were significantly lower than those produced by the roots that were inoculated with fungal treatments. This proved the success of fungal inoculation. No specific pattern regarding the lesion width and depth and occlusion width and depths was observed. As the area of the sapwood at cross section is limited, conclusions regarding the response of families to the fungal inoculation were drawn based on lesion length, lesion area, and occlusion length like Singh et al. (2014) and Matusick et al. (2010).

Lesion size determines the tolerance and susceptibility of the host (Matusick & Eckhardt, 2010; Singh et al., 2014; Stephen & Paine, 1985). The introduction of ophiostomatoid fungi in *P. taeda* induces ethylene production which further regulates monoterpene production and guides the lesion formation (Popp, Johnson, & Lesney, 1995). Paine, Raffa and Harrington (1997) further concluded that higher monoterpene accumulation denotes elevated plant defence 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 larger lesion response requires higher carbon allocation at the inoculation site (Guérard, Maillard, Bréchet, Lieutier, & Dreyer, 2007). Lahr and Krokene (2013) suggested that trees with larger necrotic lesions (more susceptible trees) have a greater decline in phloem non-structural carbohydrates and sapwood lipids. Thus, with an increasing lesion size response, there is a decrease in the radial growth (Krokene et al., 2008), loss of conductive tissue (Joseph et al., 1998; Oliva, Stenlid, & Martínez-Vilalta, 2014), xylem disruption and immediate mortality (Tyree & Zimmermann, 2002). We suggest that

TABLE 4 Estimates of differences in response variables between families inoculated with *Leptographium terebrantis* and *Grosmannia huntii* in spring 2016

Fungi	Family	Lesion area (mm^2)		Lesion length (mm)		Lesion width (mm)		Occlusion length (mm)		Occlusion depth (mm)	
		Estimate	*p*-value	Estimate	*p*-value	Estimate	*p*-value	Estimate	*p*-value	Estimate	*p*-value
LT	T1 vs T2	−129.41 (758.05)	.86	−10.27 (16.43)	.53	−1.30 (4.44)	.77	39.73 (26.47)	.14	−0.73 (1.16)	.53
	T1 vs S1	−25,359.26 (742.07)	.002	−21.10 (16.08)	.19	−15.50 (4.35)	.0006	−0.91 (25.90)	.97	−1.03 (1.13)	.37
	T1 vs S2	−4,095.43 (736.60)	<.0001	−69.23 (15.96)	<.0001	−20.34 (4.32)	<.0001	−0.11 (25.71)	.99	−1.69 (1.13)	.14
	T2 vs S1	−2,229.84 (741.85)	.003	−10.82 (16.06)	.50	−14.20 (4.35)	.0015	−40.63 (25.90)	.12	−0.30 (1.13)	.79
	T2 vs S2	−3,966.01 (755.51)	<.0001	−58.95 (16.37)	.0005	−19.04 (4.43)	<.0001	−39.84 (26.38)	.13	−0.96 (1.15)	.41
	S1 vs S2	−1,736.17 (740.80)	.02	−48.12 (16.05)	.004	−4.85 (4.34)	.27	0.79 (25.86)	.98	−0.66 (1.13)	.56
GH	T1 vs T2	−333.94 (501.45)	.51	−1.07 (11.81)	.93	−2.70 (3.61)	.46	−66.07 (36.49)	.07	0.44 (1.47)	.76
	T1 vs S1	−1,344.11 (519.85)	.01	−9.81 (12.25)	.43	−9.37 (3.74)	.01	5.12 (37.83)	.90	−3.39 (1.53)	.03
	T1 vs S2	−1,952.93 (503.35)	.0002	−35.55 (11.86)	.004	−17.17 (3.63)	<.0001	−119.15 (36.63)	.001	−0.51 (1.48)	.73
	T2 vs S1	−1,011.17 (519.50)	.05	−8.75 (12.23)	.48	−6.68 (3.74)	.07	−71.12 (36.77)	.06	−3.84 (1.53)	.01
	T2 vs S2	−1,619.99 (504.50)	.002	−34.50 (11.88)	.005	−14.76 (3.64)	.0001	−53.13 (36.71)	.15	−0.96 (1.48)	.52
	S1 vs S2	−608.82 (525.46)	.25	−25.74 (12.40)	.04	−7.80 (3.79)	.04	−124.26 (38.23)	.002	2.88 (1.54)	.07

T1 and T2: families tolerant to root-infecting ophiostomatoid fungi at the seedling stage and S1 and S2: families susceptible to root-infecting ophiostomatoid fungi at the seedling stage. LT: *Leptographium terebrantis* and GH: *Grosmannia huntii*. Estimates followed by standard errors in parenthesis. *p*-values show significant differences at $\alpha = .05$.

the tolerant families T1 and T2 inhibit the fungi at the expense of less carbon and are thus less susceptible to damage 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, Menard, & Gray, 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, Kane, & Mitton, 2014). It is, however, unclear whether either the variation in resin constituents among the different families or the variation in resin duct length and area among the families inhibits the fungal growth. The amount of production, the rate of flow and the concentration of chemical content are heritable traits that guide the resin defence in pine trees (Chhatre, Byram, Neale, Wegrzyn, & Krutovsky, 2013; Westbrook et al., 2013). The chemicals and their relative concentration which guide the levels of susceptibility and tolerance of *P. taeda* families to ophiostomatoid fungi are still unknown.

Relatively larger lesions were observed in spring inoculations compared to that of the summer (Figure 2). Several studies suggest the timing of fungal inoculation influences the response of trees (Matusick et al., 2010; Paine, 1984; Stephen & Paine, 1985), and their results greatly support our findings. Resin production is highest in the root cells formed immediately following the meristem differentiation during spring (Berryman, 1972). Thus, *P. taeda* families are more susceptible to ophiostomatoid fungal infection during the growing season (spring) (Matusick et al., 2010; Stephen & Paine, 1985).

In conclusion, this article presents the first study to show intraspecies variation in disease tolerance exists in mature *P. taeda* trees. Our results suggest that the susceptibility and tolerance to *L. terebrantis* and *G. huntii* are an inherent property of families, regardless of the age 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 defence strategies will improve our understanding of these findings.

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