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Intraspecific response of *Pinus taeda* L. to *Grosmannia huntii* and *Leptographium terebrantis* infection

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Abstract

We examined intraspecific and inter-year variation in tolerance of *Pinus taeda* to two ophiostomatoid fungi, *Leptographium terebrantis* and *Grosmannia huntii*. Containerized seedlings of *P. taeda* from 27, 32, 17 and 23 different elite genetic families were artificially inoculated with *L. terebrantis* and *G. huntii* in years 2013, 2014, 2016 and 2017, respectively. Six connector families were inoculated every year. Eight weeks post-inoculation, lesion and occlusion were measured on each seedling to determine the relative susceptibility/tolerance of families to these fungi. *Pinus taeda* families widely differed in these parameters suggesting intraspecific variation in the susceptibility/tolerance to the inoculated pathogens. The overall tolerance of the connector families to these fungi varied among the experimental years. These results showed that intraspecific variation to *L. terebrantis* and *G. huntii* exists among *P. taeda* families and it could be possible to select tolerant families to minimize the potential impact due to these fungi.

KEYWORDS
disease susceptibility, *Leptographium* species, ophiostomatoid fungi, *Pinus taeda*

1 | INTRODUCTION

Pinus taeda L. (Loblolly pine) is an important commercial *Pinus* species in the southern United States (U.S.) (Schultz, 1997). This species alone accounts for over 50% of the total softwood volume grown in this region (Oswalt, Smith, Miles, & Pugh, 2014). The number of *P. taeda* seedlings planted in the southern U.S. each year reaches a billion (McNabb & Enebak, 2008). *Pinus taeda* plantations provide marketable forest products, habitat for wildlife, and place for recreational activities and thus contribute a considerable portion of the southern U.S. economy (Poudel, Munn, & Henderson, 2017; Schultz, 1997).

Unfortunately, over the past 40 years, there have been reports of Pine Decline (PD) in the southern U.S. Pine Decline is a decline disease syndrome first reported by Brown and Mc Dowell (1968) at Talladega National Forest, Oakmulgee Ranger District, Alabama, U.S. in 1959. The decline was indicated by short chlorotic needles, sparse crowns, reduced radial growth and premature mortality. Subsequent reports of decline urged scientists to conduct further studies that revealed the association of beetle-vectored ophiostomatoid fungi with PD (Hess, Otroana, Jones, Goddard, & Walkinshaw, 1999; Hess et al., 2002). Consistent isolation of ophiostomatoid fungi: *Leptographium terebrantis* S.J. Barras and T.J. Perry, *Grosmannia huntii* R.C. Rob. Jeffr, *L. procerum* Kendrick M.J. Wingfield and *Grosmannia alacris* T.A. Duong, Z.W. de Beer and M.J. Wingfield, from the roots of declining trees (Eckhardt, Weber, Menard, Jones, & Hess, 2007) emphasizes the role of fungi in decline process, thus warranting further controlled experimental studies incorporating *P. taeda* and fungi. *Leptographium* spp. and *Grosmannia* spp. are distributed worldwide as pathogens of conifers (Jacobs & Wingfield, 2001). In North America, *L. terebrantis* and *G. huntii* are relatively more problematic (Devkota, Enebak, & Eckhardt, 2018; Matusick & Eckhardt, 2010; Wingfield, Capretti, & McKenzie, 1988).

Leptographium terebrantis with produces abundant dark mononematous conidiophores that give rise to a series of branching

metulae. The conidiophores have conidiogenous cells at the terminal end which produce single-celled pigmented hyaline conidia. *Grosmannia huntii* have distinct serpentine hyphae which initially grow hyaline and turns olivaceous with time. It produces sparse conidiophores in culture. The conidiophore gives rise to ovoid conidia. Conidia of these fungi are ideally suited for dispersal by bark-beetles as they accumulate in a slimy mass at the top of the conidiophore (Jacobs & Wingfield, 2001). Once the fungi are inoculated into the host tree during feeding activity of bark-beetle, resin-soaking, sapwood discoloration, and lesions in the phloem are observed as one of the immediate effects (Devkota, Mensah, Nadel, Matusick, & Eckhardt, 2018; Goodsman, Lusebrink, Landhäusser, Erbilgin, & Lieffers, 2013; Rice & Langor, 2008). Resin-soaking and fungal spread in the vascular tissues disturb tree water transport (Joseph, Kelsey, & Thies, 1998). In addition, investment of tree in defense may occur at the expense of radial growth (Krokene, Nagy, & Solheim, 2008).

Various *Pinus* species have shown intraspecific variation in tolerance to other tree pathogens and prompted the launch of tree breeding initiatives. For instance, open-pollinated families of *Pinus thunbergii* Parl., and *P. densiflora* Sieb. et Zucc., inoculated with a pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle in Japan (Akiba et al., 2012) exhibited intraspecific variation. Use of tolerant families in the breeding programme has resulted in 92 clones of *P. densiflora* and 16 clones of *P. thunbergi*. Similarly, *P. sylvestris* L. (Scots pine) had intraspecific variation in susceptibility to dothistroma needle blight caused by fungus *Dothistroma septosporum* (Dorog.) Morelet. with implications for more tolerant families in breeding programmes (Fraser, Brown, & Woodward, 2015). Matusick, Eckhardt, and Somers (2010) reported interspecific variation in response of *Pinus* species to *Leptographium* and *Grosmannia* species with *P. taeda* being relatively more susceptible to fungal infection than *P. palustris* and *P. elliottii*. Furthermore, Singh, Anderson, and Eckhardt (2014) artificially inoculated seedlings from a few *P. taeda* families with same fungi and presented intraspecific variation in disease tolerance. The intraspecific variation in tolerance of *P. taeda* to *L. terebrantis* and *G. huntii* is independent of the tree age and the mature tree families may have similar relative tolerance as compared to the seedling families (Devkota, Nadel, & Eckhardt, 2018).

The variation in susceptibility to pathogen observed in a few families (Singh et al., 2014) cannot be generalized to the whole population. However, despite this knowledge, and the enormous threat that the ophiostomatoid fungi pose to *P. taeda*, the question of whether intraspecific variation in tolerance/susceptibility to ophiostomatoid fungi occurs remains unexplored in many *P. taeda* families. The aims of this study thus were as follows: (a) to determine the intraspecific variation in tolerance of commonly out-planted *P. taeda* in the southern U.S. to *L. terebrantis* and *G. huntii* and (b) to understand the yearly intraspecific variation in response of *P. taeda* to *L. terebrantis* and *G. huntii*.

2 | MATERIALS AND METHODS

2.1 | Experimental design

An artificial fungal inoculation trial was conducted for 4 years. Container-grown seedlings from 27, 32, 17 and 23 different *P. taeda* families were studied in the years 2013, 2014, 2016 and 2017, respectively. The genetic distinction among groups is based on the female parent, so the term "family" is utilized. Each family was assigned a random name and original name of the families is not disclosed. Families L05, L09, L16, L38, L49 and L50 were included each year and served as connector families. The genetic distinction between these families is unknown, but families L49 and L50 represent the wild-type families. Families used belong to the most commonly out-planted half-sib (open-pollinated), or full-sib (controlled-pollinated) *P. taeda* families in the southern U.S. These families were derived from the tree genetic improvement programmes conducted by North Carolina Tree Improvement Cooperative. Each of these P. taeda families has unique characteristics which is not disclosed in this study. Each year, seeds of all test families were collected from different forest companies and send to a forest company nursery for sowing. Seedlings were raised in different nurseries each year. Plastic molded blocks containing cavities filled with growing medium were utilized to grow seedlings. Nine-month-old containerized seedlings extracted from individual containers were used in the experiment.

The study site is an outdoor research facility of the School of Forestry and Wildlife Sciences, Auburn University, located in Auburn, Alabama. To reduce individual seedling variability, the seedlings with an approximate height of 30 cm and root collar diameter (RCD) of 4.5 mm were chosen. The seedlings were planted in plastic pots (diameter-16.19 cm × height-18.41 cm) filled with ProMIx BX® (Premier Tech, Quebec, Canada) peat-based potting media in the first week of January. Randomized complete block design with six blocks was established with the random assignment of families and inoculation treatments within each block. Seedlings were allowed to acclimatize in the ambient climatic condition at the experimental site for 2 months prior to commencement of stem inoculations. Seedlings were irrigated as required to keep the soil moist.

2.2 | Inoculation of fungi

Single spore isolates of *L. terebrantis* (ATCC accession no. MYA-3316) and *G. huntii* (ATCC accession no. MYA-3311) maintained at 4°C in Forest Health Dynamics Laboratory at Auburn University, AL, U.S. were used for the stem inoculations. These isolates were sub-cultured in malt extract agar (MEA), two weeks before the start of the stem inoculation. The *L. terebrantis* and *G. huntii* isolates were isolated from the lateral roots of *P. taeda* showing symptoms of PD from the Talladega National Forest, AL, U.S. and Fort Benning Military Reservation, GA, U.S., respectively, by Eckhardt et al. (2007).

Seedlings in blocks one and three, two and five, and four and six were inoculated on March 15, April 1 and April 15, respectively. In each block, there were 28 seedlings per family. Seven seedlings per family in each block were randomly assigned to each of the following four inoculation treatments: (a) wound (control), (b) wound + sterile media (control), (c) wound + media with *L. terebrantis*, and (d) wound + media with *G. huntii*. Inoculations were performed as described by Singh et al. (2014). To perform inoculation, an 11-mm vertical wound (<2 mm deep) was created in the root collar area (2 cm above the soil line) with a sterile razor blade. Wound control received a sterile cut only. Wound + media control received a sterile agar plug in the wound. Media with fungus treatment received a 3-mm agar plug with actively growing fungal mycelium taken from the edge of the agar plate, inoculated (fungus-side-down) in the wound. Inoculation points were covered with sterile moist cotton balls to prevent desiccation of the fungal media and wrapped with Parafilm® to prevent further contamination.

2.3 | Measurements

Seedling height and RCD were measured on individual seedlings prior to stem inoculations and at harvest. Eight weeks after inoculations, seven seedlings/family/block were clipped at the soil level and placed in a tub that contained a solution of Fast-Green stain (FastGreen FCF; Sigma Chemical Co., St. Louis, MO, U.S.) and distilled water mixed in a ratio of 0.25 g/L. Seedlings were exposed to the solution for 72 hrs to allow the capillary movement of dye through the stem.

Seedlings were removed from the Fast-Green solution, and the bark tissue of each seedling was carefully scraped with a sterile razor blade to expose the lesion. The dark brown dead tissue section around the inoculation site was considered lesion length. Stems were segmented at many points away from and around the inoculation point to expose the tissue that failed to take Fast-Green dye. The length of the tissue lacking capillary action of dye was recorded as the occlusion length as described by Devkota and Eckhardt (2018).

To verify that the observed infection was caused by the same fungus inoculated, one centimetre of stem surrounding the lesion was removed from the stem and plated in MEA amended with 800 mg/L of cycloheximide and 200 mg/L of streptomycin sulphate. Plates were incubated at room temperature for 14 days, and fungal recovery from each stem piece was identified and scored.

2.4 | Statistical analyses

Mixed models were used to analyse the lesion and occlusion data with family and treatment as fixed effects and the block as a random effect. PROC MIXED statement was used in SAS 9.4. The data were checked for normality and homogeneity of variance, and log transformations were performed for yearly lesion length for the connector families. Chi-square test was performed to determine the seedling survivability. Multiple comparison tests were performed using the Tukey–Kramer test at a 5% significance level. Graphs were created in STATISTICA 10. The data were analysed using a mixed model. This model has both fixed and random effects. The statistical model used was.

$$Y_{ijk} = \mu + \mathrm{Cov} + T_i + B_j + F_k + FT + E_{ijk} \quad (1)$$

where, Y_{ijk} = response variable (for example: lesion length, occlusion length), μ = mean of parameter, Cov = initial root collar diameter of seedling as a covariate, T_i = fixed effect of treatments in block j (i = 1(*G. huntii*), 2 (*L. terebrantis*), 3 (Wound + sterile media), 4 (Wound)), B_j = random effect associated with block (j = 1..6), F_k = fixed effects of family (k = 1..n),FT = interaction effect of loblolly pine family and treatments, and E_{ijk} = residual with mean zero and constant variance (random error).

3 | RESULTS

During all study years, *G. huntii* and *L. terebrantis* led to dark brown lesions and vascular occlusion in the stems of inoculated *P. taeda* seedlings. The effect of controls, wound and wound + media was however significantly reduced as compared to the fungal inoculated seedlings and this was consistent in seedlings from all the experimental years and families. So, the effect of the controls was removed from the model.

3.1 | Year 2013

Post-fungal inoculation seedling survival was significantly different among the families tested (χ^2 = 68.3, p < 0.0001) and among the four inoculation treatments (χ^2 = 1,419.86, p < 0.0001). However, the seedling survival was not different between the seedlings receiving different inoculations within a family. The success of re-isolation of *L. terebrantis* and *G. huntii* from the inoculated seedlings was 98% and 96%, respectively.

The average lesion length caused by both fungal treatments was significantly different on various *P. taeda* families (Table 1). *Leptographium terebrantis* caused longer lesions than those by *G. huntii* (p < 0.0001). Family L73 had the shortest lesion and families L68 and L66 had the longest lesions when treated with *L. terebrantis*. Whereas families L51 and L73 had the shortest lesions and L55, L66 and L67 had the longer lesions when treated with *G. huntii* (Table 2). The occlusion length produced as a result of *L. terebrantis* inoculation was significantly higher than that produced by *G. huntii* (p < 0.0001).

3.2 | Year 2014

In 2014, survival of the inoculated seedlings was significantly different among the families (χ^2 = 188.32, p < 0.0001) but not inoculation treatments (χ^2 = 4.29, p = 0.2321). The re-isolation success of *L. terebrantis* and *G. huntii* was from the inoculated seedlings ranged from 62% to 82%. Consistent re-isolation of the fungi proved the success of the fungal inoculation.

TABLE 1 Type three fixed effects of lesion and occlusion length in study years 2013, 2014, 2016 and 2017

Year	Variable	Source	*df*	*F* value	*Pr* > *F*
2013	Lesion length	Fam	32	2.92	<0.0001
		Trt	1	504.33	<0.0001
		Fam × Trt	32	1.53	0.0295
	Occlusion length	Fam	32	2.37	<0.0001
		Trt	1	352.39	<0.0001
		Fam × Trt	32	1.42	0.0612
2014	Lesion length	Fam	37	3.94	<0.0001
		Trt	1	211.05	<0.0001
		Fam × Trt	37	1.09	0.3241
	Occlusion length	Fam	37	2.93	<0.0001
		Trt	1	383.9	<0.0001
		Fam × Trt	37	1.25	0.1462
2016	Lesion length	Fam	22	3.14	<0.0001
		Trt	1	95.17	<0.0001
		Fam × Trt	22	1.94	0.0055
	Occlusion length	Fam	22	4.03	<0.0001
		Trt	1	375.35	<0.0001
		Fam × Trt	22	2.27	0.0007
2017	Lesion length	Fam	28	1.79	0.0073
		Trt	1	33.35	<0.0001
		Fam × Trt	28	1.21	0.2094
	Occlusion length	Fam	28	1.54	0.0358
		Trt	1	18.06	<0.0001
		Fam × Trt	28	1.13	0.2923

Notes. df: Degree of freedom; Fam: Family; Trt: Fungal treatment.

Grosmannia huntii produced significantly longer lesion length than *L. terebrantis* ($p < 0.0001$). Similarly, occlusion length caused by *G. huntii* was significantly longer than that caused by *L. terebrantis* ($p < 0.0001$) (Table 1). Lesion length and occlusion length were significantly different among the families. However, family and fungal interaction was not statistically significant for both lesion length and occlusion length. Families L108 and L99 had shorter lesions and L81 and L91 had most extended lesions when treated with *L. terebrantis* (Table 3). Whereas families L86 and L108 had the shortest lesions and families L88 and L91 had the longest lesions when treated by *G. huntii*.

3.3 | Year 2016

The seedling survival was not significantly different among the family. All families had 100% seedling survival except families L50, L114 and L127 which had 97% survival rate. Neither *G. huntii* nor *L. terebrantis* inoculation affected seedling survival. The success of re-isolation of *G. huntii* and *L. terebrantis* was 96% and 93%, respectively, from the inoculated seedlings.

Lesion length and occlusion length differed significantly between two fungal treatments ($p = <0.0001$) and families ($p = <0.0001$) (Table 1). The fungal treatment and family interaction were significant for lesion length ($p = 0.002$) and occlusion length ($p = <0.0001$). Families, L126, L130 and L129 had the longest, and L118 and L09 had the shortest lesion length when treated with *G. huntii*. Families, L126 and L129 had the longest average lesion length, and L33 and L111 had the shortest lesion length when challenged with *L. terebrantis* (Table 4).

3.4 | Year 2017

In 2017, the two fungi did not cause significantly different lesion length in loblolly pine seedlings from the same family. So, the family can be ranked based on overall fungal inoculation or two separate fungi. The post-inoculation seedling survival was 100%. The success of re-isolation of *L. terebrantis* and *G. huntii* was 76% and 50%, respectively. Families L133 and L131 had the shortest, and families L38 and L151 had the longest lesion length when treated with *L. terebrantis*. Similarly, families L50 and L16 had the shortest and families L143, L146, and L149 had the longest lesion length when treated with *G. huntii* (Table 5).

3.5 | Connector families

The six connector families responded similarly to the fungal treatments in experimental years, 2013 and 2014 (Figure 1). However, the lesion length of the overall connector families varied by year of

TABLE 2 Least square means and standard errors of the lesion length caused by overall fungi, *Leptographium terebrantis* and *Grosmannia huntii* in *Pinus taeda* families in year 2013

Family	Overall–LL LS mean ± *SE* (mm)	LT–LL LS mean ± *SE* (mm)	GH–LL LS mean ± *SE* (mm)
L66	35.84 ± 1.43a	42.09 ± 2.23a	29.27 ± 1.32a
L68	34.88 ± 1.44a	42.45 ± 2.32a	27.67 ± 1.30ab
L67	34.80 ± 1.42ab	39.67 ± 2.21ab	29.57 ± 1.32a
L56	34.39 ± 1.43abc	41.41 ± 2.29a	27.54 ± 1.30ab
L55	33.48 ± 1.43abcd	38.02 ± 2.26abcd	28.83 ± 1.32ab
L05	32.80 ± 1.45abcd	40.25 ± 2.32ab	25.53 ± 1.32abc
L09	32.13 ± 1.54abcde	38.61 ± 2.56abc	26.67 ± 1.35ab
L38	32.13 ± 1.44abcde	39.22 ± 2.32ab	25.38 ± 1.30abc
L77	32.08 ± 1.43abcde	38.49 ± 2.35abc	26.42 ± 1.27ab
L54	31.90 ± 1.51abcde	39.07 ± 2.56abc	26.31 ± 1.30ab
L59	31.56 ± 1.54bcde	37.22 ± 2.48abcd	26.22 ± 1.39ab
L62	31.47 ± 1.42abcde	34.91 ± 2.26abcde	28.11 ± 1.29ab
L57	31.31 ± 1.44abcde	36.94 ± 2.26abcde	25.40 ± 1.34abc
L76	31.02 ± 1.43abcde	37.38 ± 2.29abcd	24.82 ± 1.30abcd
L69	30.86 ± 1.46abcde	38.22 ± 23.8abcd	24.22 ± 1.30bcd
L16	30.75 ± 1.48abcde	36.17 ± 2.41abcde	25.86 ± 1.32abc
L65	30.53 ± 1.46abcde	36.01 ± 2.35abcde	25.33 ± 1.32abc
L60	30.07 ± 1.44abcde	32.96 ± 2.26cde	27.03 ± 1.34ab
L50	29.93 ± 1.46abcde	36.12 ± 2.41abcde	24.63 ± 1.29abcd
L64	29.89 ± 1.43abcde	34.29 ± 2.32abcde	25.90 ± 1.27abc
L58	29.81 ± 1.43abcde	36.21 ± 2.41abcde	24.68 ± 1.24abcd
L49	29.61 ± 1.43abcde	33.55 ± 2.29bcde	25.76 ± 1.30abc
L63	29.57 ± 1.46abcde	34.24 ± 2.38abcde	25.36 ± 1.30abc
L74	29.56 ± 1.50abcde	34.61 ± 2.60abcde	25.92 ± 1.27abc
L53	29.39 ± 1.47bcde	34.53 ± 2.41abcde	24.88 ± 1.30abcd
L51	29.24 ± 1.44bcde	36.38 ± 2.41abcde	23.39 ± 1.26cd
L71	29.18 ± 1.43bcde	33.77 ± 2.32bcde	24.93 ± 1.29abcd
L72	28.61 ± 1.45cde	32.24 ± 2.32de	25.06 ± 1.32abc
L61	28.48 ± 1.43de	32.47 ± 2.23cde	24.18 ± 1.34bcd
L70	28.48 ± 1.43de	31.14 ± 2.23e	25.68 ± 1.32abc
L75	27.68 ± 1.42de	31.26 ± 2.28e	24.35 ± 1.27abcd
L52	27.64 ± 1.40de	30.57 ± 2.21e	24.65 ± 1.29abcd
L73	26.00 ± 1.38e	28.35 ± 2.13e	23.43 ± 1.29cd

Notes. Different letters indicate Tukey's Honest significant differences between *Pinus taeda* families within each fungal treatment at $\alpha = 0.05$.
GH: *Grosmannia huntii*; LL: Lesion length; LT: *Leptographium terebrantis*; SE: Standard error.

inoculation ($F_{3,1693} = 312.40$, $p = <0.0001$). The lesion length was shortest in the year 2016 and longest in the year 2013.

4 | DISCUSSION

There was intraspecific variation in tolerance/susceptibility (regarding lesion length and occlusion length) of *P. taeda* to *L. terebrantis* and *G. huntii*. Similar variation was observed in response of *Pinus* families to *Fusarium circinatum* (Roux et al., 2007), and clones of *Ulmus americana* L. (American elm) to *Ophiostoma ulmi* (Buism) (Tchernoff, 1965). Jankowiak, Banach, and Balonek (2013) reported similar response of *Quercus robur* L. (pedunculate oak) families to *Phytophthora cambivora* (Petri) Buisman. Current screening trials show that there is significant potential for selecting PD tolerant *P. taeda* from current southeastern U.S. planting stock. These families have the potential for use as parents in breeding programmes to maximize the disease tolerance in *P. taeda* and thus to ensure that losses due to fungi associated with PD can be minimized in the future.

TABLE 3 Least square means and standard errors of the lesion length caused by overall fungi, *Leptographium terebrantis* and *Grosmannia huntii* in *Pinus taeda* families in year 2014

Family	Overall–LL LS mean ± *SE* (mm)	LT–LL LS means ± *SE* (mm)	GH–LL LS means ± *SE* (mm)
L81	37.39 ± 1.77a	36.11 ± 2.58a	38.67 ± 2.24a
L91	36.72 ± 1.68a	33.73 ± 2.43ab	39.80 ± 2.14a
L87	33.01 ± 1.61ab	28.08 ± 2.31ab	38.34 ± 2.09a
L102	32.44 ± 1.54ab	29.33 ± 2.25ab	35.55 ± 1.96abc
L83	32.44 ± 1.56b	27.51 ± 2.25b	37.61 ± 2.01ab
L80	32.31 ± 1.58b	28.02 ± 2.25ab	37.07 ± 2.06ab
L78	31.92 ± 1.64b	26.65 ± 2.37bc	37.49 ± 2.11ab
L88	31.81 ± 1.57b	24.12 ± 2.31bcd	39.31 ± 1.98a
L82	31.81 ± 1.77b	26.95 ± 2.71bc	35.84 ± 2.14abc
L104	30.99 ± 1.74bc	24.11 ± 2.58bcd	37.47 ± 2.18ab
L93	30.75 ± 1.58bc	25.68 ± 2.34bcd	35.57 ± 1.98abc
L90	30.56 ± 1.59bc	25.70 ± 2.37bcd	35.07 ± 1.98abc
L109	30.41 ± 1.47bc	25.58 ± 2.15bcd	35.24 ± 1.87abc
L96	30.40 ± 1.57bc	25.22 ± 2.28bcd	35.71 ± 2.01abc
L97	30.01 ± 1.61bc	26.45 ± 23.4bc	33.67 ± 2.06bcd
L100	29.62 ± 1.62bc	23.49 ± 2.40bcd	35.44 ± 2.03abc
L103	29.18 ± 1.66bcd	24.70 ± 2.43bcd	33.55 ± 2.09bcd
L79	28.96 ± 1.56bcd	26.07 ± 2.31bc	31.71 ± 1.96bcd
L49	28.83 ± 1.64bcd	27.55 ± 2.40b	30.11 ± 2.09bcd
L106	28.65 ± 1.57bcd	23.91 ± 2.28bcd	33.50 ± 2.01bcd
L101	28.15 ± 1.58bcd	24.31 ± 2.25bcd	32.41 ± 2.06bcd
L38	27.89 ± 1.53cd	25.25 ± 2.31bcd	30.25 ± 1.89bcd
L92	27.87 ± 1.69cd	24.26 ± 2.50bcd	31.28 ± 2.11bcd
L98	27.52 ± 1.59cd	27.00 ± 2.34b	28.02 ± 2.01cd
L107	27.47 ± 1.55cd	24.64 ± 2.25bcd	30.36 ± 1.98bcd
L09	27.45 ± 1.49cd	23.83 ± 2.15bcd	31.24 ± 1.91bcd
L89	27.37 ± 1.57cd	23.23 ± 2.28bcd	31.60 ± 2.01bcd
L94	27.13 ± 1.58cd	25.27 ± 2.31bcd	28.98 ± 2.01cd
L84	26.98 ± 1.86cd	22.99 ± 2.76bcd	30.70 ± 2.32bcd
L05	26.91 ± 1.55cd	24.03 ± 2.25bcd	29.86 ± 1.98bcd
L50	26.81 ± 1.69cd	26.38 ± 2.40bc	27.29 ± 2.21cd
L16	26.53 ± 1.56cd	24.28 ± 2.28bcd	28.77 ± 1.98cd
L85	26.47 ± 1.58cd	23.13 ± 2.37bcd	29.49 ± 1.96bcd
L110	26.42 ± 1.81cd	25.77 ± 2.86bcd	26.89 ± 2.14cd
L95	26.02 ± 1.56cd	23.18 ± 2.31bcd	28.72 ± 1.96cd
L86	24.93 ± 1.56d	23.67 ± 2.31bcd	26.13 ± 1.96cd
L99	24.87 ± 1.56d	21.76 ± 2.28cd	27.97 ± 1.98cd
L108	23.07 ± 1.56e	19.85 ± 2.31e	26.14 ± 1.96cd

Notes. Different letters indicate Tukey's Honest significant differences between *Pinus taeda* families within each fungal treatment at $\alpha = 0.05$.
GH: *Grosmannia huntii*; LL: Lesion length; LT: *Leptographium terebrantis*; SE: Standard error.

Pinus taeda families with shorter lesion were considered relatively tolerant to the fungi than the families with longer lesions. On an ecological scale, the bark-beetle and the associated fungi (a) must overcome the tree defense, and (b) obtain food from the tree. The utilization of the tree's resources such as sapwood resources and non-structural carbohydrates (NSC) by the trees in defense may lead to depletion of the resources. The relatively tolerant families can defend against the fungi by utilizing fewer resources. In the susceptible family, the successfully colonized ophiostomatoid fungi use the tree's resources, and the resources

TABLE 4 Least square means and standard errors of the lesion length caused by overall fungi, *Leptographium terebrantis* and *Grosmannia huntii* in *Pinus taeda* families in year 2016

Family	Overall–LL LS mean ± *SE* (mm)	LT–LL LS mean ± *SE* (mm)	GH–LL LS mean ± *SE* (mm)
L38	25.99 ± 1.99a	26.84 ± 2.67a	24.94 ± 2.87ab
L16	25.99 ± 2.05a	23.40 ± 2.69abc	28.66 ± 3.03a
L113	24.58 ± 1.99a	24.27 ± 2.64ab	24.78 ± 2.90ab
L129	24.53 ± 1.99a	25.11 ± 2.64b	23.79 ± 2.90ab
L126	24.33 ± 1.97ab	24.47 ± 2.64ab	24.11 ± 2.84ab
L124	24.10 ± 2.00ab	25.45 ± 2.67a	22.55 ± 2.90bc
L49	23.90 ± 1.98ab	23.30 ± 2.61abc	22.72 ± 2.93bc
L114	23.59 ± 1.99ab	24.43 ± 2.67ab	22.61 ± 2.87bc
L122	23.24 ± 1.99ab	25.42 ± 2.64a	20.80 ± 2.90cd
L09	23.15 ± 1.98ab	22.73 ± 2.61bc	21.76 ± 2.93bc
L33	23.05 ± 2.02ab	24.19 ± 2.67abc	21.67 ± 2.96bc
L111	22.58 ± 1.98abc	24.80 ± 2.64ab	20.14 ± 2.87cd
L118	22.55 ± 1.99abc	21.77 ± 2.64bcd	23.26 ± 2.90ab
L123	22.49 ± 1.99abc	24.37 ± 2.64ab	20.38 ± 2.90cd
L116	22.39 ± 1.98bc	25.02 ± 2.64ab	19.55 ± 2.87de
L112	22.31 ± 2.01bc	24.27 ± 2.69ab	20.20 ± 2.90cd
L127	22.22 ± 1.97bc	23.11 ± 2.59bc	21.13 ± 2.90cd
L130	21.82 ± 1.98bcd	21.44 ± 2.61bcd	22.17 ± 2.90bc
L117	21.52 ± 1.97bcd	21.87 ± 2.61bcd	21.07 ± 2.87cd
L115	20.82 ± 1.98bcd	20.79 ± 2.64cd	20.79 ± 2.87cd
L05	20.52 ± 1.95cd	22.03 ± 2.59bc	18.89 ± 2.84de
L50	19.87 ± 2.01d	19.67 ± 2.67d	20.03 ± 2.93cd
L128	19.47 ± 1.97d	20.17 ± 2.59cd	18.64 ± 2.90de

Notes. Different letters indicate Tukey's Honest significant differences between *Pinus taeda* families within each fungal treatment at $\alpha = 0.05$.
GH: *Grosmannia huntii*; LL: Lesion length; LT: *Leptographium terebrantis*; SE: Standard error.

decline over time impacting the growth and development of the tree. The trees with relatively larger lesions as a response to fungal inoculation have greater resource reduction (Lahr & Krokene, 2013).

As indicated by the relatively longer lesion and occlusion length, *L. terebrantis* and *G. huntii* were found to be relatively more pathogenic in the year 2013 than in years, 2014, 2016 and 2017. Our results are similar to those of Singh et al. (2014) where they reported that the pathogenicity of the fungi varied among years. Singh et al. (2014) gave two possible explanations for this variation: (a) use of different seedling stocktypes in different years or (b) genotype × environment interaction. The former reason can be excluded as we utilized containerized seedlings only. Thus, genotype × environment interaction might have resulted in differences in fungal pathogenicity among years. In this regard, in January 2013 (when seedlings were potted), the monthly average temperature was 12°C (according to Auburn, Alabama weather underground). In contrast, in January 2014, the average temperature was 3°C, respectively. In addition, seedlings were subjected to a winter storm after planting. Similarly, the average monthly temperatures were 8°C and 6°C during January 2016 and 2017, respectively. Thus, the observed differences in fungal pathogenicity between years could be due to family × environment interaction as the slight difference in the temperature might have caused the alteration in the seedling susceptibility and fungal pathogenicity. Also, the seedlings were grown in different nurseries each year. Variation in the microclimate and cultural practices among the nurseries might have played a role in causing the variation in the results between the years. Future studies should be conducted to explore the relative variation in fungal virulence and susceptibility of *P. taeda* families to these fungi at varying temperatures and other environmental conditions.

The two ophiostomatoid fungi varied in virulence among each year of inoculation. In 2013 and 2016, *L. terebrantis* was found to be more virulent than *G. huntii*. Whereas, in 2014 (when the seedling growing condition was cold), *G. huntii* was relatively more virulent (in terms of lesion length) than *L. terebrantis*. Similar to our findings, Matusick and Eckhardt (2010) reported *G. huntii* was more virulent than *L. terebrantis* in *Pinus* species in the southern U.S. Although we lack experiments with controlled temperature and fungal virulence, results suggest the disease-causing ability of the pathogen is associated with either how stressed the hosts are due to adverse environmental condition or how conducive is

TABLE 5 Least square means and standard errors of the lesion length caused by overall fungi, *Leptographium terebrantis* and *Grosmannia huntii* in *Pinus taeda* families in year 2017

Family	Overall–LL LS mean ± *SE* (mm)	LT–LL LS mean ± *SE* (mm)	GH–LL LS mean ± *SE* (mm)
L38	20.56 ± 0.55a	22.70 ± 0.81a	18.42 ± 0.73ab
L149	19.88 ± 0.57ab	20.33 ± 0.89a	19.51 ± 0.73a
L146	19.34 ± 0.55ab	19.26 ± 0.83ab	19.42 ± 0.72a
L151	19.31 ± 0.55ab	20.66 ± 0.79a	17.85 ± 0.75abc
L142	19.30 ± 0.54ab	19.56 ± 0.79ab	19.04 ± 0.72a
L143	19.23 ± 0.56ab	18.94 ± 0.84abc	19.51 ± 0.73a
L05	19.19 ± 0.55ab	19.45 ± 00.81ab	18.93 ± 0.73ab
L09	19.12 ± 0.56ab	19.71 ± 0.83ab	18.52 ± 0.75ab
L134	19.09 ± 0.56ab	19.32 ± 0.83ab	18.87 ± 0.73ab
L147	19.06 ± 0.54ab	19.55 ± 0.81ab	18.59 ± 0.72ab
L140	18.95 ± 0.54ab	19.13 ± 0.81ab	18.77 ± 0.72ab
L139	18.91 ± 0.57abc	19.29 ± 0.87ab	18.57 ± 0.75ab
L137	18.74 ± 0.54abc	19.20 ± 0.79ab	18.26 ± 0.73ab
L135	18.70 ± 0.56abc	19.57 ± 0.83ab	17.88 ± 0.73abc
L148	18.67 ± 0.57abc	18.95 ± 0.84abc	18.40 ± 0.77ab
L145	18.67 ± 0.56abc	18.83 ± 0.84abc	18.52 ± 0.73ab
L150	18.51 ± 0.54abc	19.14 ± 0.81ab	17.90 ± 0.72abc
L136	18.46 ± 0.55abc	18.37 ± 0.81bc	18.55 ± 0.73ab
L153	18.41 ± 0.54abc	18.96 ± 0.81abc	17.89 ± 0.72abc
L152	18.34 ± 0.54abc	18.95 ± 0.81abc	17.76 ± 0.72abc
L132	18.34 ± 0.57abc	19.18 ± 0.83ab	17.41 ± 0.78abc
L144	18.32 ± 0.55abc	19.12 ± 0.83ab	17.59 ± 0.72abc
L49	18.25 ± 0.56abc	19.22 ± 0.81ab	17.19 ± 0.77bc
L141	18.20 ± 0.54abc	18.26 ± 0.79bc	18.15 ± 0.72ab
L138	18.13 ± 0.55abc	18.53 ± 0.81abc	17.73 ± 0.73abc
L16	18.03 ± 0.57abc	19.09 ± 0.83ab	16.91 ± 0.77bc
L50	17.98 ± 0.54c	19.28 ± 0.79ab	16.62 ± 0.73bc
L131	17.90 ± 0.54c	18.31 ± 0.79bc	17.49 ± 0.72abc
L133	17.82 ± 0.53cd	18.12 ± 0.79bc	17.54 ± 0.73abc

Notes. Different letters indicate Tukey's Honest significant differences between *Pinus taeda* families within each fungal treatment at $\alpha = 0.05$.
GH: *Grosmannia huntii*; LL: Lesion length; LT: *Leptographium terebrantis*; SE: Standard error.

the condition for the growth of pathogen (Stenlid & Oliva, 2016). Our results underline the need to include the role of the environment while predicting the impact of the invasive pathogens (Dukes et al., 2009).

Families utilized in the present study have the desired attributes (undisclosed) depending on the objective of the forest companies. These families responded differently to the *L. terebrantis* and *G. huntii*, whereas the wild-type families had the intermediate levels of fungal tolerance. This suggests that different families chosen for desired attributes may differ in their tolerance towards the ophiostomatoid fungi. Thus, a particular attribute such as growth phenology, wood density, wood volume, etc. may or may not benefit the tree against the attack by the studied fungi. Wild-type families may be relatively tolerant to these fungi than some of the susceptible families but use of wild-type families may not meet the objective of the timber companies in the southern U.S. Screening o f P. taeda families to these fungi helps in selection of tolerant families which can further be utilized in tree breeding and improvement programs. In a broader sense, results from this study reveal the necessity of tree breeding programs to consider pest and pathogen tolerance attributes of the trees while breeding trees for other desired traits.

In conclusion, *P. taeda* families show wide variation in response to ophiostomatoid fungi associated with the PD thus indicating family genetics play an essential role in the variation in response to the fungi. Pathogenicity of the two fungi varies even within a particular family so relative tolerance of *P. taeda* families to *L. terebrantis* and *G. huntii* should be considered separately. Future studies should focus on screening disease tolerance of mature tree families on field settings and on understanding anatomical and chemical defense mechanisms that govern tolerance of specific *P. taeda* families to these fungi.

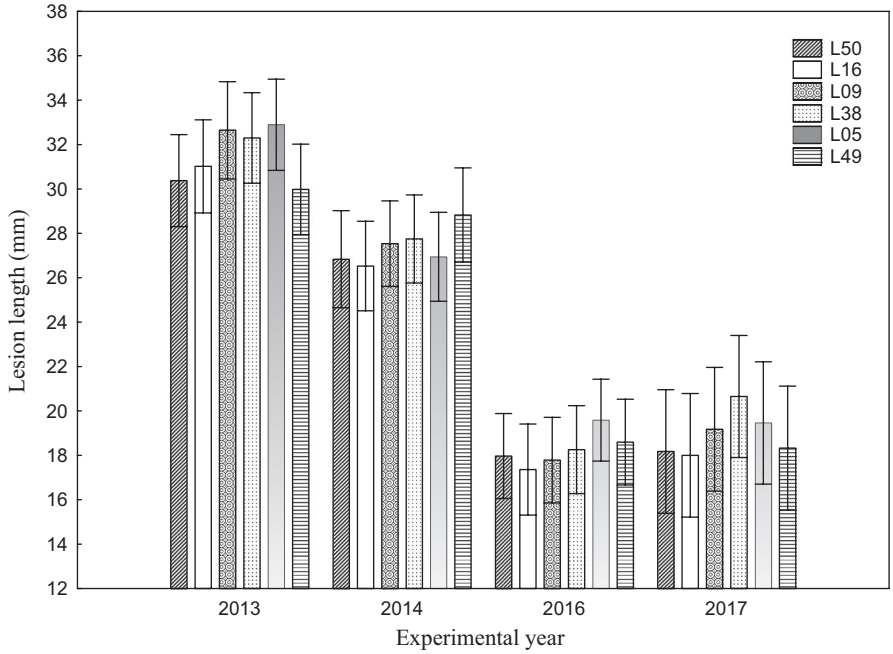

FIGURE 1 Means of lesion length caused by *Leptographium terebrantis* and *Grosmannia huntii* in six different *Pinus taeda* families in years, 2013, 2014, 2016 and 2017

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