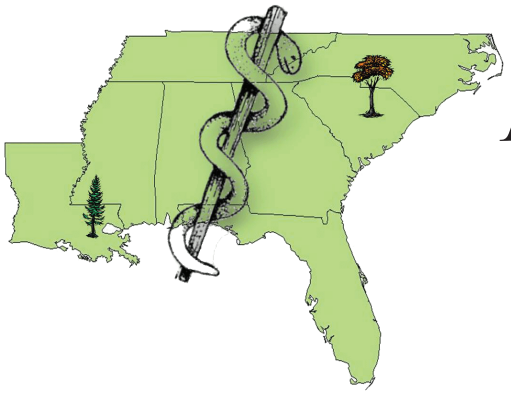

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# RESEARCH REPORT 17-05

## INTRASPECIFIC AND INTER-STOCKTYPE RESPONSE OF *PINUS TAEDA* L. TO *GROSMANNIA HUNTII* AND *LEPTOGRAPHIUM TEREBRANTIS*

by
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**ABSTRACT**
Vascular-inhabiting ophiostomatoid fungi are among the contributing factors of premature decline and mortality of *Pinus taeda* (loblolly pine). The aim of this experiment was to examine intraspecific variation in tolerance of *P. taeda* to ophiostomatoid fungi. Containerized and bare-root seedlings for 94 and 4 families respectively, were artificially inoculated at the stem with *L. terebrantis* and *G. huntii*. Eight weeks post inoculation, lesion and occlusion were measured on each seedling to determine variation of host responses. *Pinus taeda* showed wide intraspecific variation in tolerance/susceptibility to both *L. terebrantis* and *G. huntii*. The two interspecies stocktypes (bare-root and container) of *P. taeda* had similar tolerance to fungi. Results suggest both seedling stocktypes can be used in virulence screening studies. *Pinus taeda* families more tolerant to ophiostomatoid fungi can be separated from less tolerant. These results will help land managers in making decisions to plant most appropriate *P. taeda* families to minimize the potential impact of ophiostomatoid fungi.

**INTRODUCTION**
*Pinus taeda* L. (loblolly pine) is the leading pine species which comprises 50% of the total softwood volume grown in the south (Schultz, 1997; Oswalt et al., 2014). The number of *P. taeda* seedlings planted in the southern U.S. each year reaches a billion (McNabb and Enebak, 2008). *Pinus taeda* plantations provide marketable forest products, provides habitat for wildlife, and place for recreational activities and thus contribute a considerable portion of the southern U.S. economy (Poudel et al., 2017; Schultz, 1997). Over the past 40 years, however, there have been reports of Pine Decline.

Pine Decline (PD), a decline disease syndrome, first reported by Brown and Mc Dowell (1968) at Talladega National Forest, Oakmulgee Ranger District 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 et al., 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 et al., 2007) emphasizes the role of fungi in decline process, thus warranting further controlled experimental studies incorporating *P. taeda* and fungi.

*Leptographium terebrantis* and *G. huntii*, are distributed worldwide as pathogens of conifers (Jacobs and Wingfield, 2001). Whereas, in North America, the former pathogen is relatively more

problematic (Wingfield et al., 1988; Matusick and Eckhardt, 2010). However, mature root inoculations in *P. taeda*, *P. palustris* (longleaf pine) and *P. elliottii* (slash pine) in the southern U.S. revealed relatively higher virulence of *G. huntii* than *L. terebrantis* (Matusick and Eckhardt, 2010). One of the immediate effects of *L. terebrantis* and *G. huntii* in *Pinus* hosts include resin-soaking, sapwood discoloration and lesions in the phloem (Wingfield 1986; Matusick and Eckhardt 2010; Chieppa et al., 2017).

Ophiostomatoid fungi affect pine species differently with *P. taeda* being relatively more susceptible than *P. palustris* and *P. elliotti* (Matusick et al., 2010). Singh et al. (2014) studied intra-species variation in tolerance of *P. taeda* to *L. terebrantis* and *G. huntii* and found *P. taeda* families widely varied in tolerance/susceptibility to both *Leptographium* and *Grosmannia* species. Various *Pinus* species have shown intra-species 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 et Buhrer) Nickle in Japan (Akiba et al., 2012) exhibited intra-species variation. Use of tolerant families in the breeding program has resulted in 92 clones of *P. densiflora* and 16 clones of *P. thunbergi*. Similarly, *Pinus sylvestris* L. (Scots pine) had intraspecific variation in susceptibility to dothistroma needle blight caused by fungi *Dothistroma septosporum* (Dorog.) Morlet. with implications for more tolerant families in breeding programs (Fraser et al., 2015).

Bare-root and containerized seedlings are the two stock types used in previous screening studies (Singh et al., 2014; Chieppa et al., 2017). Bare-root seedlings are grown in soil beds in an open field with the removal of soil during harvest. Containerized seedlings are grown in containers containing artificial media under a shelter or controlled greenhouse environment with root and soil media maintained together from harvest to re-planting (Grossnickle et al., 2016). The lifting of bare-root seedlings in nurseries in the southeastern U.S. involves a number of operational procedures which might affect root viability (Starkey and Enebak, 2013). In contrast, root damage is minimal in containerized seedlings. Containerized seedlings usually have higher survival success rates than bare-root after replanting (Sloan et al., 1987; South et al., 2005). However, the use of a greenhouse, growth medium, proper irrigation and fertilization may make containerized seedlings more susceptible to biotic diseases (Grossnickle et al., 2016). Singh et al. (2014) studied intraspecific variation in tolerance to ophiostomatoid fungi in either containerized or bare-root seedlings respectively in two separate years. The containerized seedlings had both higher field survival and longer lesions compared to bare-root seedlings. However, the results were inconclusive as interfamily-stocktype differences were not studied. Thus, the study of the response of two stocktypes (container-grown and bare-root seedlings) of the same families of *P. taeda* is necessary.

The variation in susceptibility to pathogen observed in few families 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 remains unexplored in many *P. taeda* families. The hypotheses of this study are: (i) *Pinus taeda* families out-planted in the southern U.S. have intra-specific variation in tolerance to two major fungi associated with PD, (ii) *Pinus taeda* stocktypes vary in tolerance to ophiostomatoid fungi, and (iii) The connector families will show a similar response to the fungal inoculation in all the three years.

## MATERIALS AND METHODS

### Experimental design

Container-grown seedlings from 33, 38 and 23 different *P. taeda* families were studied in 2013, 2014 and 2016 respectively (Table 1.1, Table 1.2 and Table 1.3). In 2014, bare-root seedlings from 4 families same as container grown families also were studied. The genetic distinction among groups is based on female parent, so the term "family" is utilized. 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 wildtype 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 programs conducted by North Carolina Tree Improvement Cooperative. Seeds from all families were grown within a single forest company nursery each year to minimize environmental variability. Nine-month-old, containerized seedlings and bare-root seedlings were extracted from individual containers and common nursery beds and used in the experiment. Twelve seedlings per family were randomly selected and separated into the stem, needles, coarse root and fine root and dried at oven (75 °C for 72 h) for initial biomass assessment.

The study site is an outdoor research facility of the School of Forestry and Wildlife Sciences, Auburn University, located in Auburn, Alabama. A randomized complete block design with six blocks (3 replications) (Figure 1.2) was established with the random assignment of families and treatments within each block. Seedlings were planted in one-gallon pots filled with ProMIx BX® (Premier Tech, Quebec, and Canada) peat-based potting media. The seedlings with a mean height of 30 cm and root collar diameter of 4.5 mm (Figure 1.1) were chosen for planting to reduce individual seedling variability. The planted seedlings were allowed to acclimatize in the ambient climate condition at the experimental site for 2 months prior commencement of stem inoculations. Seedlings were irrigated when required.

### Inoculation of fungi

Single spore isolates of *Leptographium terebrantis* (ATCC accession no. MYA-3316) and *Grosmannia 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, 2 weeks before the start of the stem inoculation. The *L. terebrantis* isolate was isolated from the lateral root of *P. taeda* in the Talladega National Forest, Oakmulgee Ranger District, AL, U.S. The *G. huntii* isolate was isolated from the lateral root of *P. taeda* from Fort Benning Military Reservation, GA, U.S. Those trees exhibited symptoms of PD such as thin crowns and lateral roots with a damaged localized root tissue as described by Eckhardt et al. (2007).

Seven seedlings (in 2013 and 2014) and 10 seedlings (in 2016) from each family received 1 of 4 inoculation treatments. The inoculation treatments were: (i) wound (control), (ii) wound + sterile media (control), (iii) wound + media with *L. terebrantis,* and (iv) wound + media with *G. huntii*. To perform inoculation, an 11-mm vertical wound (< 2 mm deep) in root-collar-area 2 cm above the soil line was created with a sterile razor blade (Figure 1.3). 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 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. Furthermore, the inoculation area was wrapped with Parafilm® to prevent further contamination.

**Measurements**

Seedling height and Root-Collar-Diameter (RCD) were measured on individual seedlings prior to stem inoculations and at harvest. During harvest, 7 seedlings/family/block were clipped at the soil level and placed in a tub that contained solution of Fast-Green stain (FastGreen FCF; Sigma Chemical Co., St. Louis, MO, USA) and distilled water mixed in a ratio of 0.25 g $L^{-1}$. Seedlings were exposed to the solution for 72 h to allow the capillary movement of dye through the stem. Three seedlings/family/block (in 2016) were gently pulled from the pot with roots and used for seedling dry matter biomass measurements as described earlier.

After removal from the Fast-Green solution, the lesion-length-width-depth, occlusion- length-width, and depth were measured on each seedling. The dark brown dead tissue section around the inoculation site was considered lesion length. Stem tissue lacking capillary action to allow Fast-Green dye to pass through it was recorded as occlusion.

To verify Koch's Postulates of disease diagnosis, one centimeter of stem surrounding the lesion was removed from the stem and plated in MEA amended with 800 mg $L^{-1}$ of cycloheximide and 200 mg $L^{-1}$ of streptomycin sulphate. Plates were incubated at room temperature for 14 days and fungal recovery from each stem piece was identified and scored.

**Statistical analyses**

Mixed-models were used to analyze 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 if there was a violation of these assumptions. Estimate statements were used to evaluate effects of each treatment. Multiple comparison tests were performed using Tukey-Kramer test at a 5% significance level. Graphs were created in STATISTICA 10.

The data were analyzed using mixed model. This model has both fixed and random effects. The statistical model used was

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

Where,

$Y_{ijk}$ = Response variable (for example : lesion length, occlusion length )

$\mu$ = Mean of parameter

$T_{ij}$ = Fixed effect of treatments in block j (i = 1(GH)..,2(LT)..,3(WM)..,4(W))

$Cov$ = Initial root collar diameter of seedling as a covariate

$B_j$ = Random Effect associated with block (j = 1..6)

$F_k$ = Fixed Effects of family (k = 1..n)

$FT_{ki}$ = Interaction effect of loblolly pine family and treatments $(GH, LT, W \text{ and } WM)$

$E_{ijk}$ = Residual with mean zero and constant variance (Random error)

Working hypotheses:

Family and inoculation treatments are under fixed effects. We want to compare whether there is significant differences in lesion length among the families.

We were mainly interested in testing family effects:

$Ho$: $\mu_1 = \mu_2 = \cdots \mu_k$ (Mean lesion length of the families are not significantly different)

$HA$: $\mu_1 \neq \mu_k$ (Mean lesion length is different in at least between two families)

Also, when inoculation treatment effects are considered:

$Ho: T_1 = T_2 = \cdots T_i = 0$ (There is no effect of the treatment on lesion development)

$Ha: T_i \neq 0$ for at least one fungal treatment

## RESULTS

### Year 2013

Both the fungi used in the inoculation trials led to dark brown lesions in seedlings form families tested (Figure 1.5). The fungal re-isolation from seedling stems was 96 to 98%. Seedling survival was significantly different among the families tested (Chi-sq = 68.36, $P$ <0.0001) and among the inoculation treatments (Chi-sq = 1419.86, $P$ <0.0001). However, the seedling survival was not different between the seedlings receiving different inoculations within a family.

The lesion length produced by the wound and wound + media was significantly smaller than that caused by the fungal treatments. So, the effect of the controls were removed from the model. The average lesion length caused by both fungal treatments on different *P. taeda* families is shown in Figure 1.6. *Leptographium terebrantis* caused longer lesions that those by *G. huntii* ($P$ <0.0001) as given in Table 1.1. Family L73 had the shortest lesions 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 S1). Occlusion observed was moving both vertically and radially. The occlusion length produced as a result of *L. terebrantis* inoculation was significantly higher than that produced by *G. huntii* ($P$ <0.0001).

Covariance parameter estimates showed that lesion length for families tested were significantly different from zero (Z = 0.02). The average overall lesion length and those caused by *G. huntii* and *L. terebrantis* is shown in Figure 1.6, 1.7, and 1.8, respectively. Lesion width was not significantly different from zero (Z = 0.19). Similarly, occlusion length (Z = 0.35) and occlusion width (Z = 0.47) was not significantly different from zero (Table 1.4).

The length, width and depth of lesions were found to be affected by fungal treatments as shown by type three fixed effects (Table 1.5). A family x treatment interaction was not found to be significant ($P$ = 0.07) which indicates within each family two fungi did not cause differing sizes of lesion. Thus, an overall ranking of families (in terms of lesion length) can be done as shown in Table S2.

### Year 2014

In 2014, the survival of inoculated seedlings was significantly different among the families (Chi-sq = 188.32, $P$ <0.0001) but was not due to inoculation treatments (Chi-sq = 4.29, $P$ = 0.2321).

Inoculation of the two fungi resulted in dark brown necrotic lesions around inoculation zone. Re-isolation of the fungi from the inoculated seedlings ranged from 62% to 82% (Table S6). Consistent re-solation of the fungi proved the success of the inoculation.

Lesion length caused by both fungal treatments were significantly longer than those resulting from the control treatments as shown by pairwise comparisons test (Table 1.6). The effects of both controls were removed, and only the effects of the fungal treatments were included in the model. *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 1.7).

Lesion and occlusion length was significantly different among the families as indicated by covariance parameter estimates (Table 1.8). However, family and treatment interaction was not statistically significant for both lesion length and occlusion length. Lesion and occlusion length, depth and width were found to be affected by fungal treatments ($P$ <0.0001) (Table 1.9). Families L108 and L99 had shorter lesions and L81 and L91 had longest lesions when treated with *L. terebrantis*. Whereas, families L86 and L108 had the shortest lesions and families L88 and L91 had the longest lesions when treated by *G. huntii*.

There was no significant difference in lesion length between the two stocktypes used in the trial when challenged with *L. terebrantis* and *G. huntii* (Figure 1.9 and 1.10). Although differences were observed among different families, none of the same two families had significantly different lesion lengths (Figure 1.9 and 1.10).

**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* ($F = 9.94$, $\chi^2 = 0.99$) nor *L. terebrantis* ($F = 3.21$, $\chi^2 = 0.67$) affected seedling survival. The re-isolation success of *G. huntii* and *L. terebrantis* was 96% and 93% respectively from the inoculated seedlings.

Inoculation of *L. terebrantis* and *G. huntii* in *P. taeda* seedlings resulted in dark brown lesions around the inoculation points. The lesions caused by both fungal treatments were significantly longer than those lesions resulting from the control treatments (Table 1.10). Lesion length, width and depth and occlusion length, width and depth differed significantly between two fungal treatments ($P$ = <0.0001) and families (P = <0.0001). The fungal treatment and family interaction was significant for lesion length ($P$ = 0.002), occlusion length ($P$ = <0.0001) and occlusion depth ($P$ = 0.0171). However, the interaction was not significant for lesion width ($P$ = 0.3784), lesion depth ($P$ = 0.3049) and occlusion width ($P$ = 0.0505) (Table 1.11). 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*.

Inoculations did not alter seedling biomass, however, biomass varied by family ($F_{(22, 1420)} = 13.40$, $P$ = <0.001). Fungal treatment interaction was not significant ($F_{(66, 1420)} = 0.93$, $P = 0.6369$)

indicating that biomass did not change in seedlings within families receiving different inoculation treatments.

**Response of connector families in all years**

The 6 connector families which were inoculated every year did not show any family x year interaction (in terms of lesion length). However, the lesion formation varied by year of inoculation. The lesion length was smallest in the year 2016 and greatest in 2014. These families did not respond differently to the fungal inoculation (in terms of lesion length) within each year. The six families responded similarly to the fungal inoculation (in terms of lesion length) in within each experimental year (Figure 1.17).

## DISCUSSION

There was intra-species variation in tolerance/susceptibility (regarding lesion length and occlusion length) of *P. taeda* to *L. terebrantis* and *G. huntii*. Similar variation was observed in the study of different *Pinus* families in response to *Fusarium circinatum* Roux et al. (2007), and clones of *Ulmus americans* L. (American elm) to *Ophiostoma ulmi* (Buism). Tchernoff (1965), reported similar response of *Quercus robur* L. (pedunculate oak) families to *Phytophthora cambivora* (Petri) Buisman. Jankowiak et al. (2013). 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 programs to maximize the disease tolerance in *Pinus taeda* and thus to ensure that losses due to fungi associated with PD can be minimized in the future.

Intraspecific variation in tolerance of the *P. taeda* to ophiostomatoid fungi is independent of the seedling growth conditions. Bare-root and container-grown seedlings demonstrated no inter-stocktype differences in response to ophiostomatoid fungi (Figure 1.17). Both stocktypes of a single family responded similarly. Containerized seedlings had higher survival rate than the bare-root, making former stocktype more favorable to use in the experimental design studies.

Lesions observed in host seedling after fungal inoculation serves as a reliable estimator of the fungal virulence as well host response (Matusick and Eckhardt, 2010, Matusick et al., 2010). While other host responses such as lesion depth, lesion width, occlusion length, and occlusion depth and occlusion width also were measured, these responses did not provide sufficient evidence to draw any conclusions regarding relative tolerance as the depth of sapwood in limited. Occlusion length acted as supporting response variable.

*Pinus taeda* families with shorter lesion were considered relatively tolerant to the fungi than the families with longer lesions. In an ecological scale, the bark-beetle and the associated fungi (i) must overcome the tree defense, and (ii) 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 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 and 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 2013 than 2014 or 2016. 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: (i) use of different seedling stocktypes in different years or (ii) genotype x environment interaction. The former reason can be excluded as our results suggest that inter-stocktype response to ophiostomatoid fungi do not exist. Thus, genotype x environment interaction might have resulted in differences in fungal pathogenicity among years. In this regard, in January 2013 (when seedlings were potted), lowest and monthly average temperature were -1 º and 12 ºC respectively (according to weather underground[1]). In contrast, in January 2014, the lowest and the average temperature was -12 ºC and 3 ºC respectively which was lower compared to 2013. In addition, seedlings were subjected to a winter storm on January 28. Thus, the observed differences in fungal pathogenicity between years could be due to family x environment interaction. This can be further supported by Petäistö et al (1993) who suggested that trees experiencing cold and frost also are more susceptible to infection by *Gremmeniella abietina*.

The 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*. Our finding that *G. huntii* is relatively virulent than *L. terebrantis* has been supported by Matusick and Eckhardt, 2010 where they reported *G. huntii* to be 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 the condition for the growth of pathogen (Stenlid and 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). Tolerance to the *L. terebrantis* and *G. huntii* varies between the families, and the findings are consistent with the previous years.

Fungi had no effect on seedling growth regarding seedling biomass. Some short-term studies have found that the growth ability of *Pinus taeda* seedling may vary between the family independent of the fungal inoculation (Chieppa et al. 2015; Chieppa et al., 2017). Some other conifer and ophiostomatoid fungal interactions studies have not considered the impact of fungi on seedling growth (Matusick and Eckhardt, 2010; Singh et al., 2014). Oliva et al., (2014) suggests that vascular-inhabiting fungi obtain sugar from degraded cells around the lesion and xylem sap in order to thrive. In addition, allocation of the more carbon in defense of vascular-inhabiting fungi cause resource starvation which decreases plant productivity (Oliva et al., 2014). In a natural scenario, bark beetles inoculate the fungi on previously stressed trees. So, it is possible that the fungi negatively impact the growth and biomass of the trees in the field at a higher level. We, therefore, suggest that long-term studies should be conducted to understand the impact of these fungi on seedling biomass.

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 virulence. This suggests

[1] http://www.wunderground.com

that different families chosen for desired attributes differ in their tolerance towards the ophiostomatoid fungi, but wildtype families do not differ. Wild-type families may be relatively less susceptible to fungi than some of the susceptible families but use of these families may not meet the objective of the timber companies in the southern U.S. Thus a particular attribute such as growth phenology, wood density, wood volume, etc. may or may not benefit the plant against the attack by the studied fungi. Thus, the selection of families tolerant to these fungi provides an opportunity to make a decision regarding the planting of the suitable *P. taeda* families.

In conclusion, *P. taeda* families show wide variation in response to ophiostomatoid fungi associated with the pine decline thus indicating family genetics play an important role in the variation in response to the fungi. Containerized and bare-root seedling stocktypes of the same family are equally susceptible or tolerant to *L. terebrantis* and *G. huntii* suggesting both types of the seedlings can be used in further screening studies. 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. Since the tolerance of the families is tested at the premature stage future, studies should be performed on mature *P. taeda* families. Also, future studies should be focused on transcriptional changes in the tolerant and susceptible families following fungal inoculation to improve our understanding of mechanisms of disease tolerance.

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**Figure 1.1** Container-grown *Pinus taeda* seedlings from a single family before re-planting.

**Figure 1.2** *Pinus taeda* seedlings planted in six blocks (RCBD) in the outdoor planting space**.**

**Figure 1.3** Artificial inoculation of fungi in the stem of *Pinus taeda*.

**Figure 1.4** *Pinus taeda* seedlings dipped in FastGreen stain after clipping.

**Figure 1.5** The dark necrotic lesions observed in *Pinus taeda* seedlings 8 weeks following inoculations.

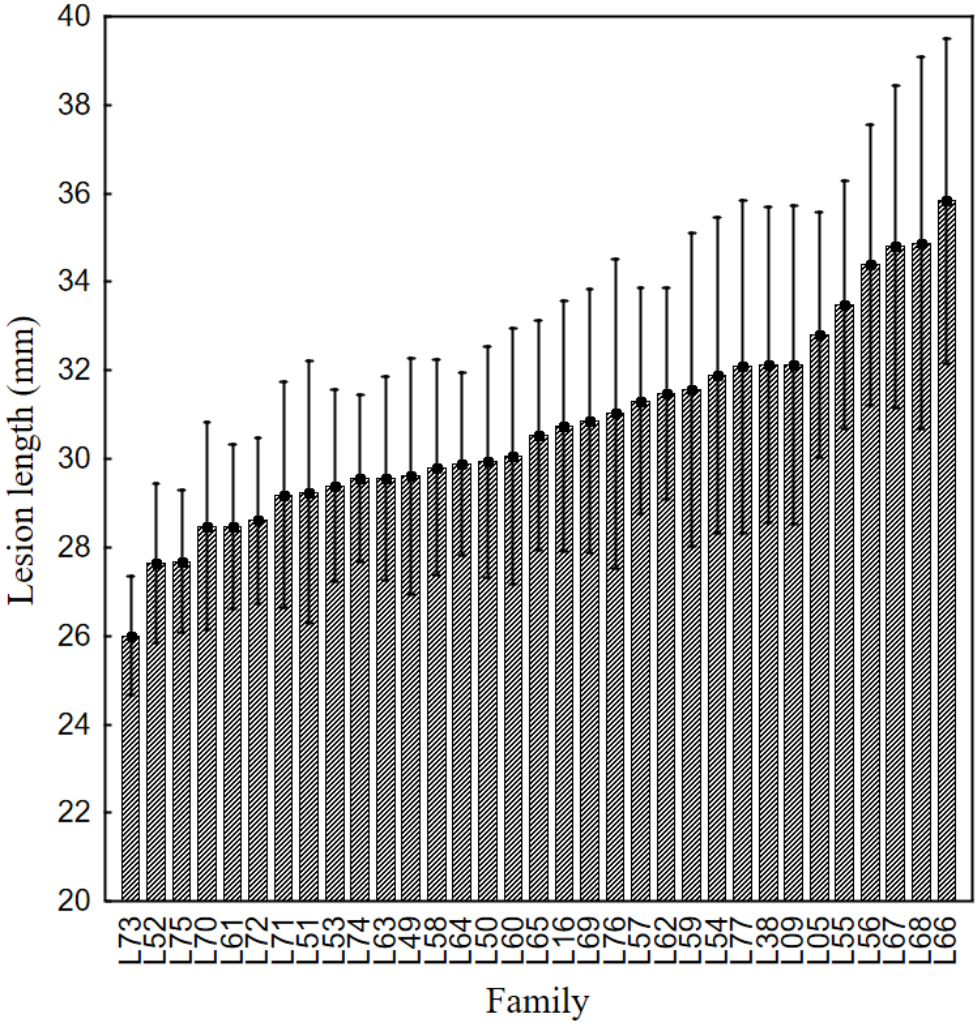


**Figure 1.6** Mean lesion length caused by the both fungal treatments in *Pinus taeda* families (year: 2013). Error bars indicate 95% confidence interval.

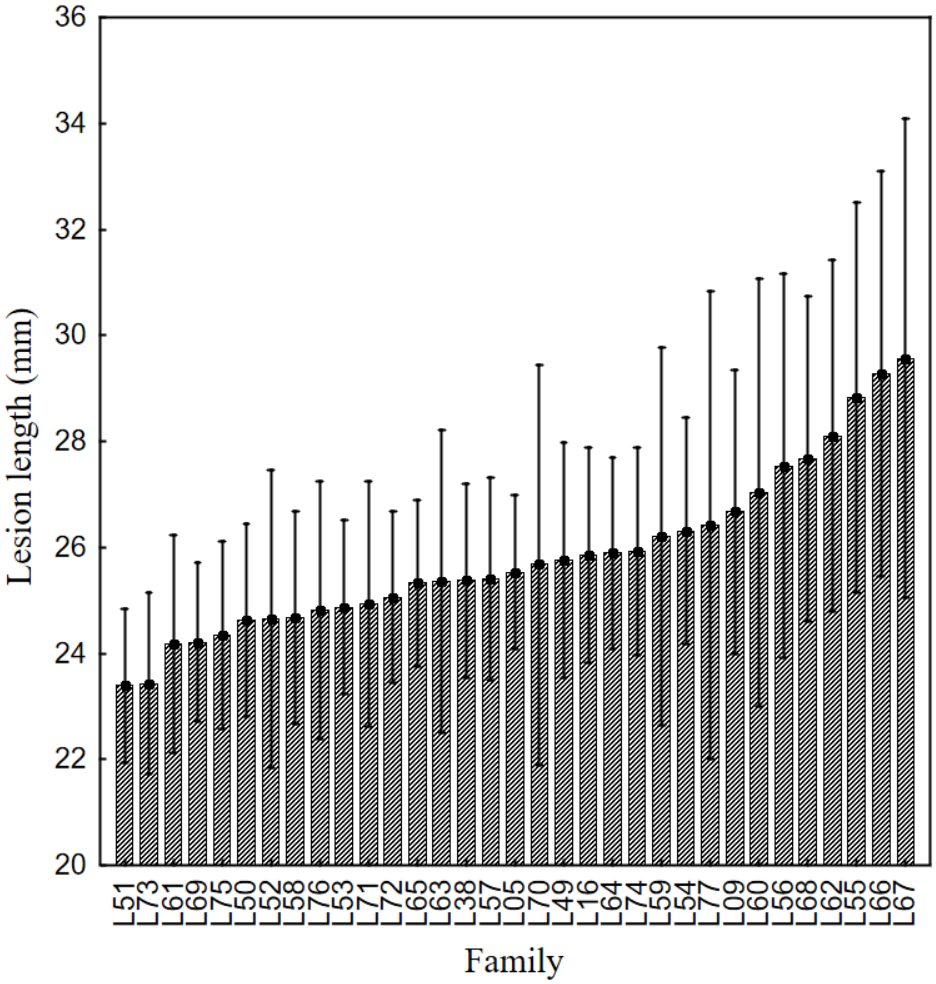


**Figure 1.7** Mean lesion length caused by *Grosmannia huntii* in different *Pinus taeda* families (year: 2013). Error bars indicate 95% confidence interval.

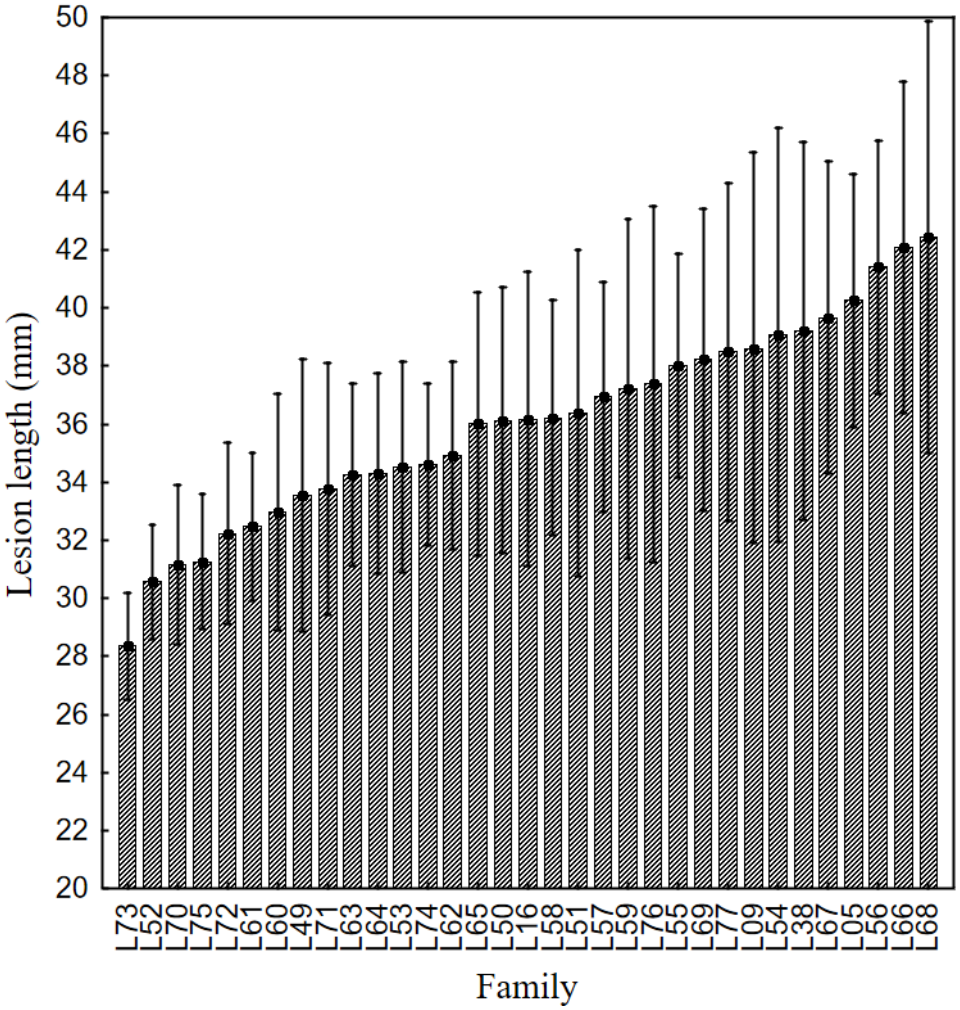


**Figure 1.8** Mean lesion length caused by *Leptographium terebrantis* in different *Pinus taeda* families (year: 2013). Error bars indicate 95% confidence interval.

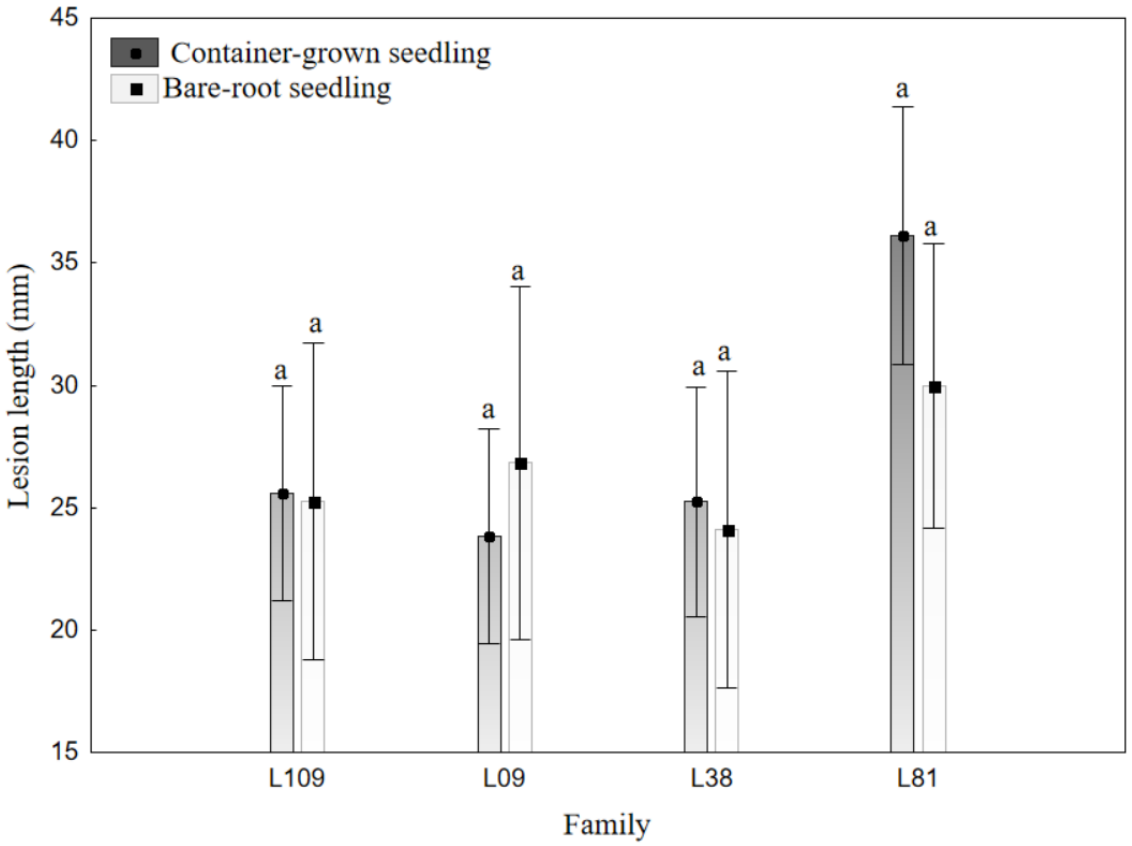


**Figure 1.9.** Mean lesion length on bare-root and container-grown families *P. taeda* families inoculated with *Leptographium terebrantis* (year: 2014). Error bars indicate 95% confidence interval. Different letters indicate significant differences between same bare-root and container-grown seedlings within same family.

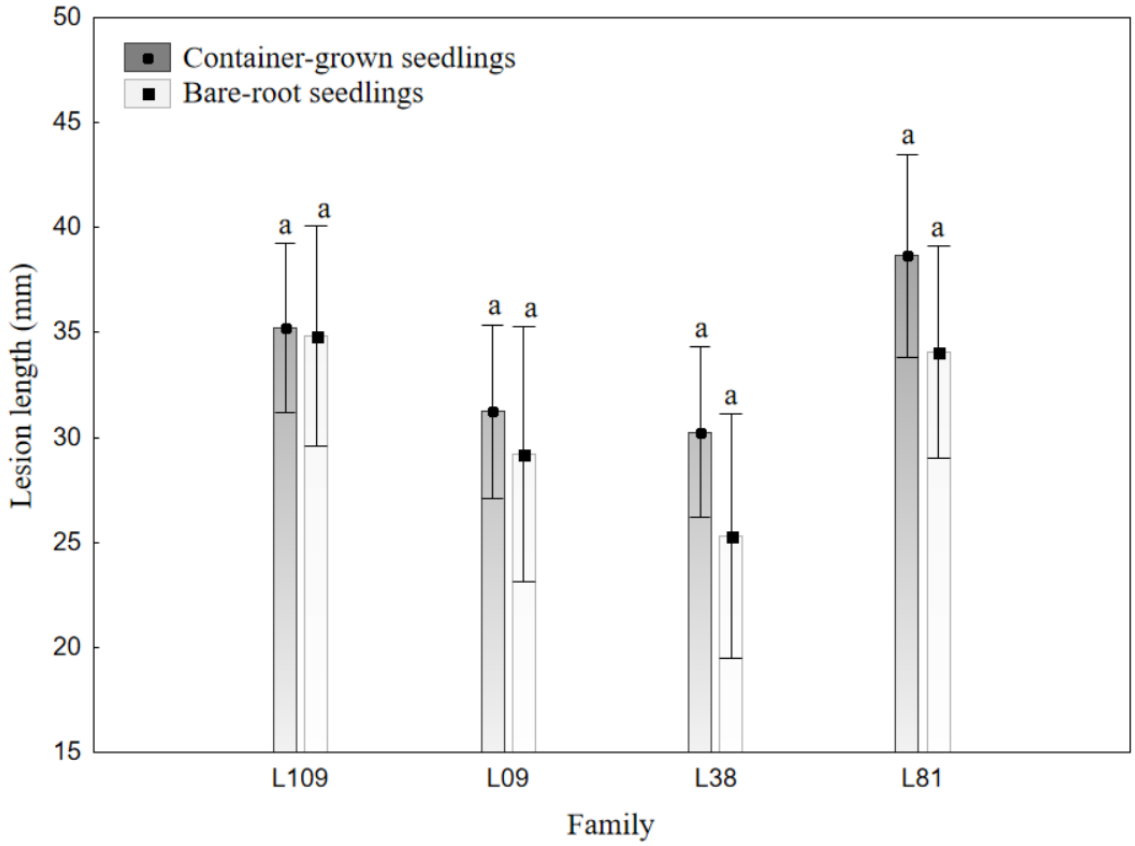


**Figure 1.10** Mean lesion length on bare-root and container-grown *Pinus taeda* families inoculated with *Leptographium terebrantis* (year: 2014). Error bars indicate 95% confidence interval. Different letters indicate significant differences between bare-root and container-grown seedlings within same family.

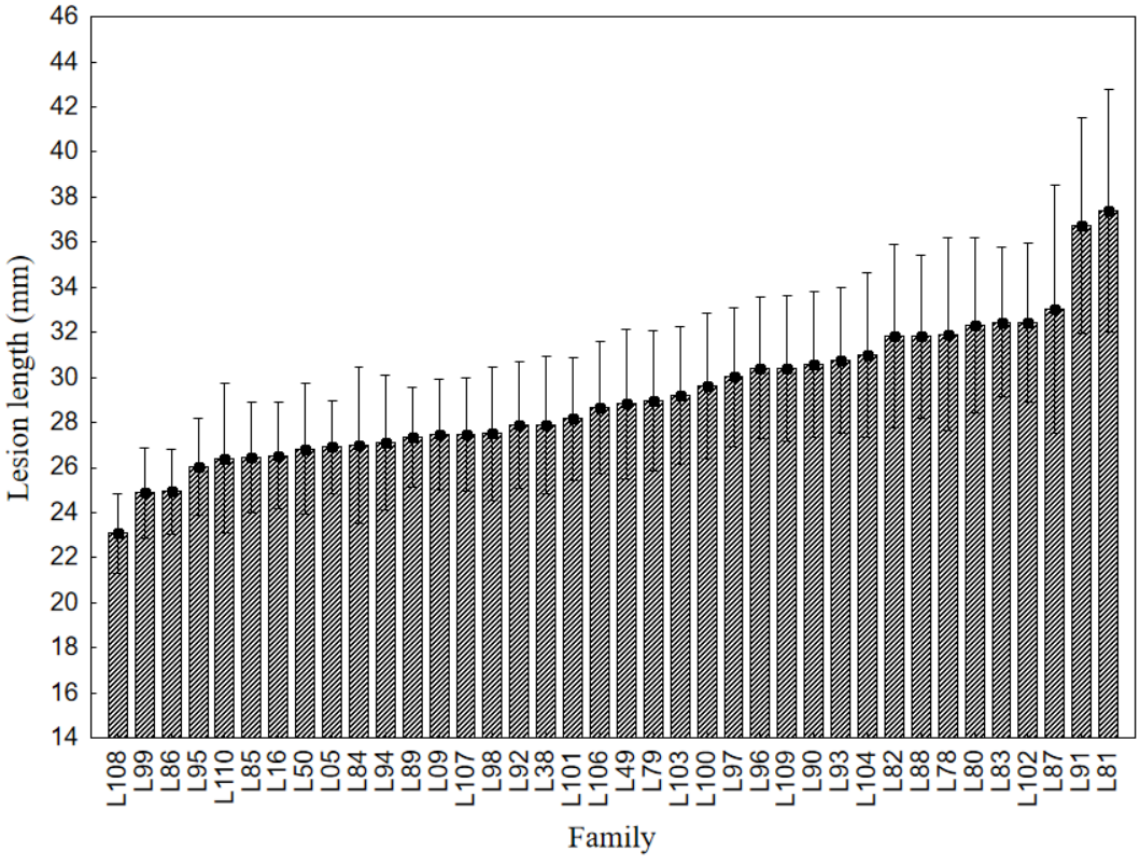


**Figure 1.11** Mean lesion length caused by both the fungal treatments on *Pinus taeda* families (year: 2014). Error bars indicate 95% confidence interval.

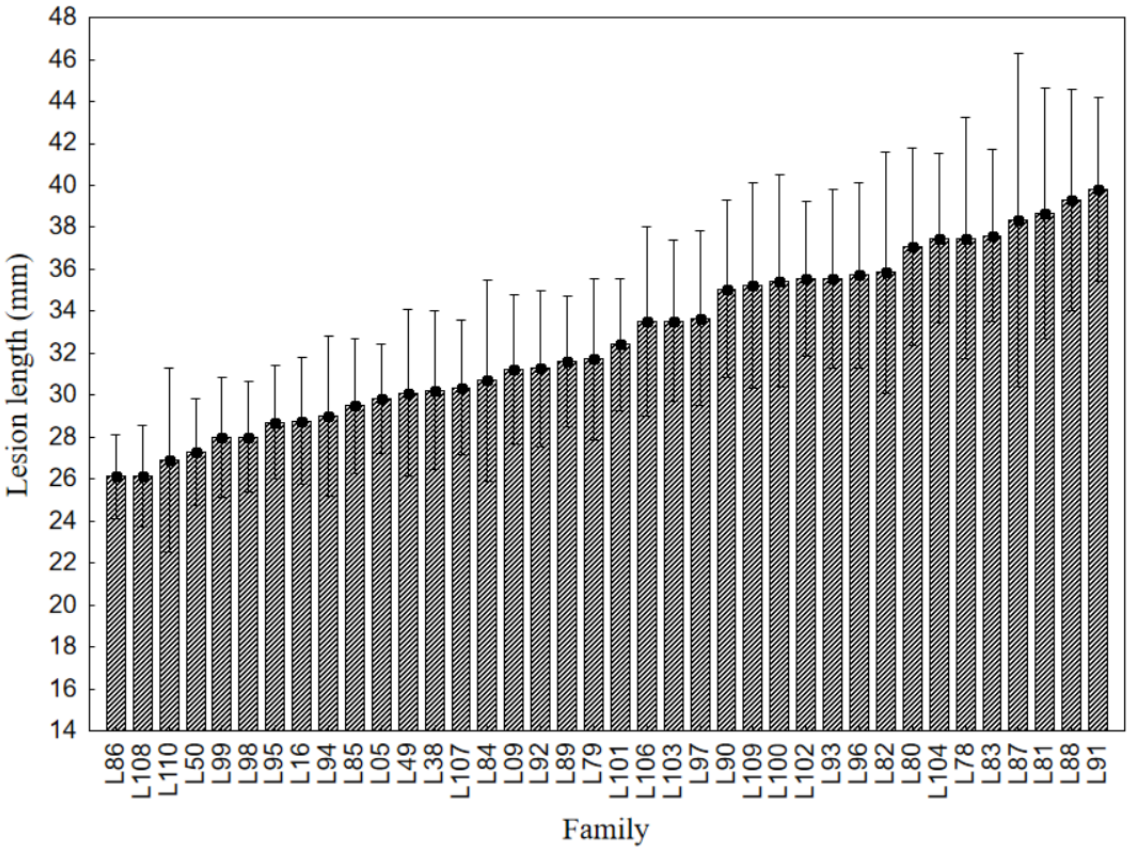


**Figure 1.12** Mean lesion length caused by *Grosmannia huntii* on different *Pinus taeda* families (year: 2014). Error bars indicate 95% confidence interval.

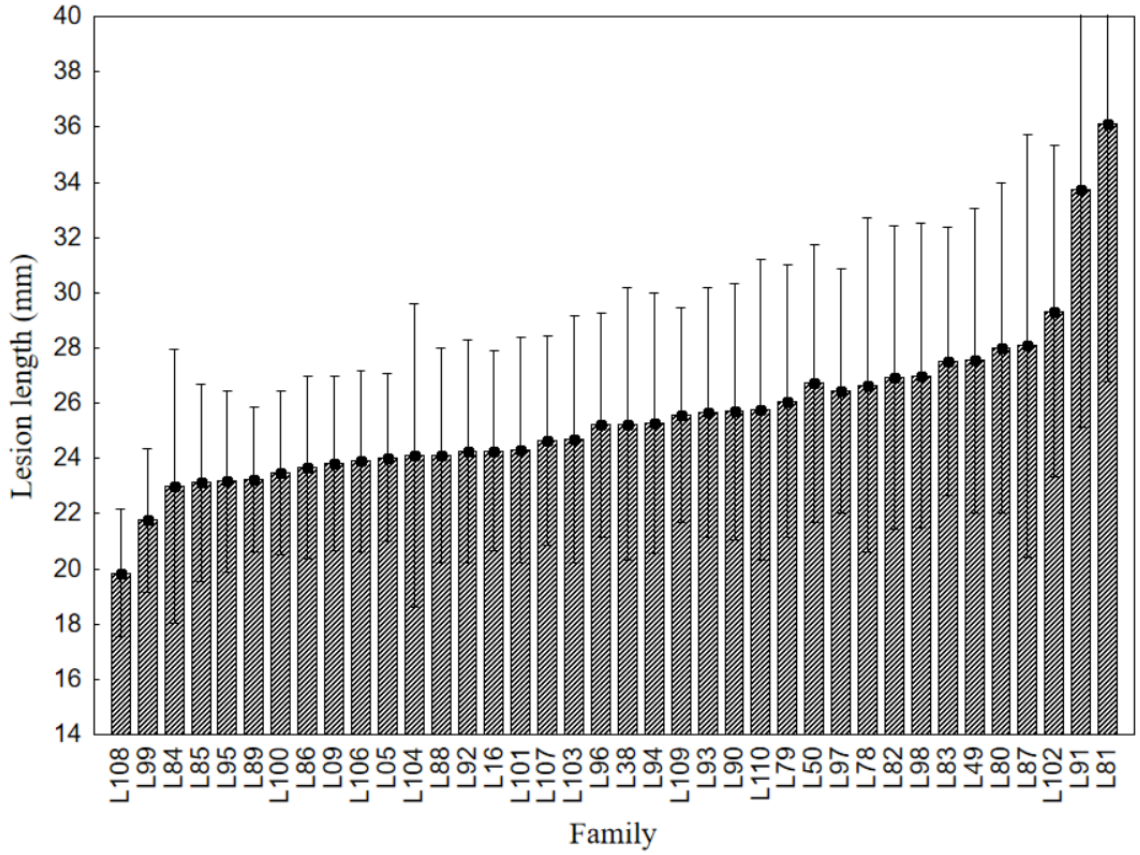


**Figure 1.13** Mean lesion length caused by *Leptographium terebrantis* on different *Pinus taeda* families (year: 2014). Error bars indicate 95% confidence interval.

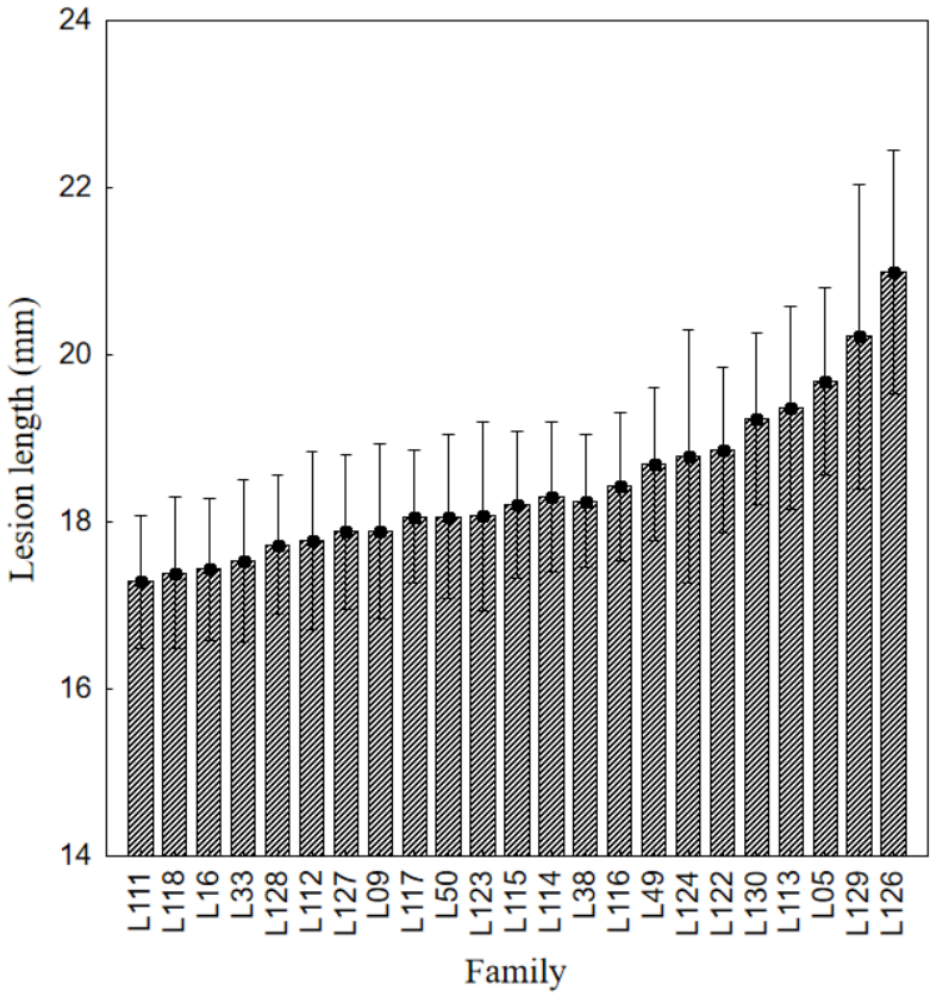


**Figure 1.14** The overall mean lesion lengths caused by both the fungal treatments on *Pinus taeda* families (year: 2016). 95% confidence intervals are indicated by error bars.

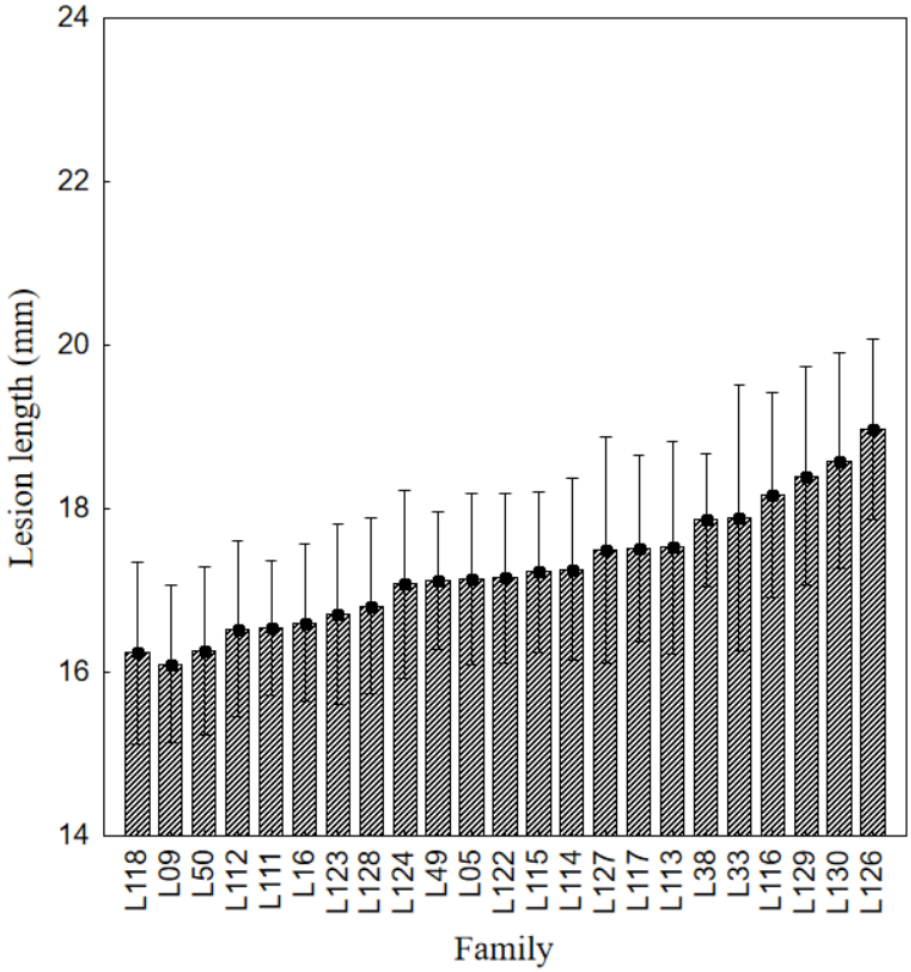


**Figure 1.15** Mean lesion length caused by *Grosmannia huntii* on *Pinus taeda* families (year: 2016). Error bars indicate 95% confidence interval.

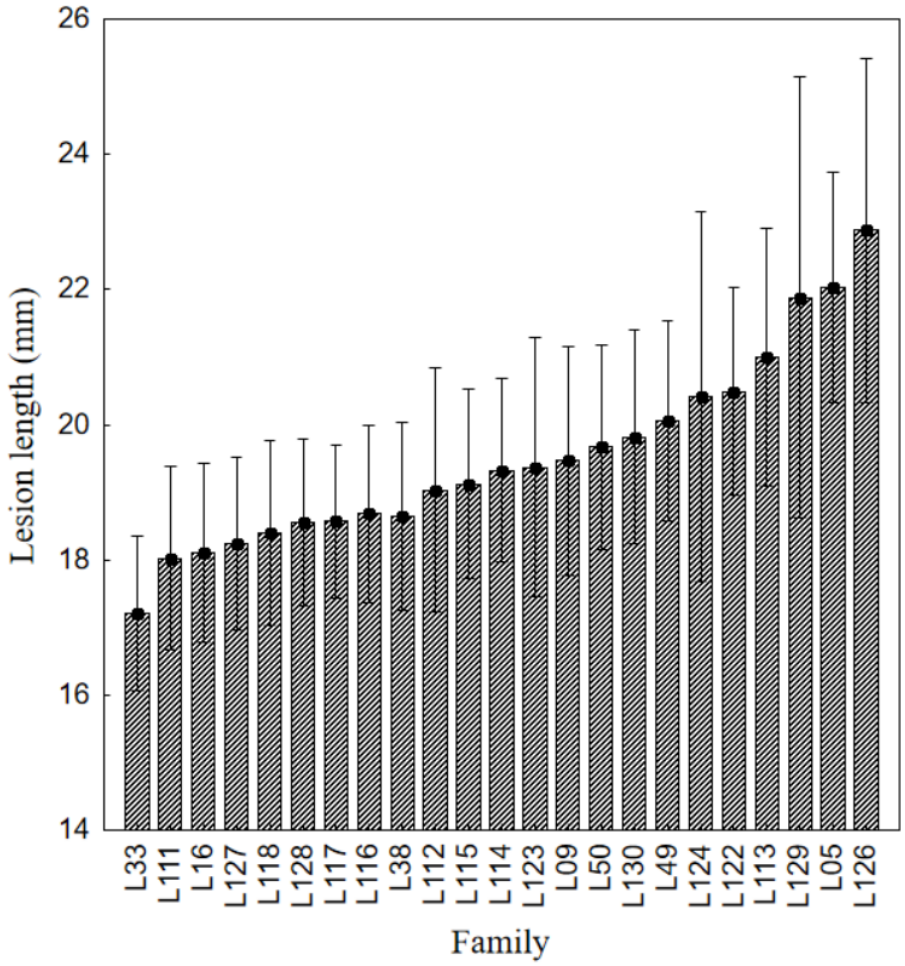


**Figure 1.16** Mean lesion length caused by *Leptographium terebrantis* on *Pinus taeda* families (year: 2016). Error bars indicate the 95% confidence interval of the mean.

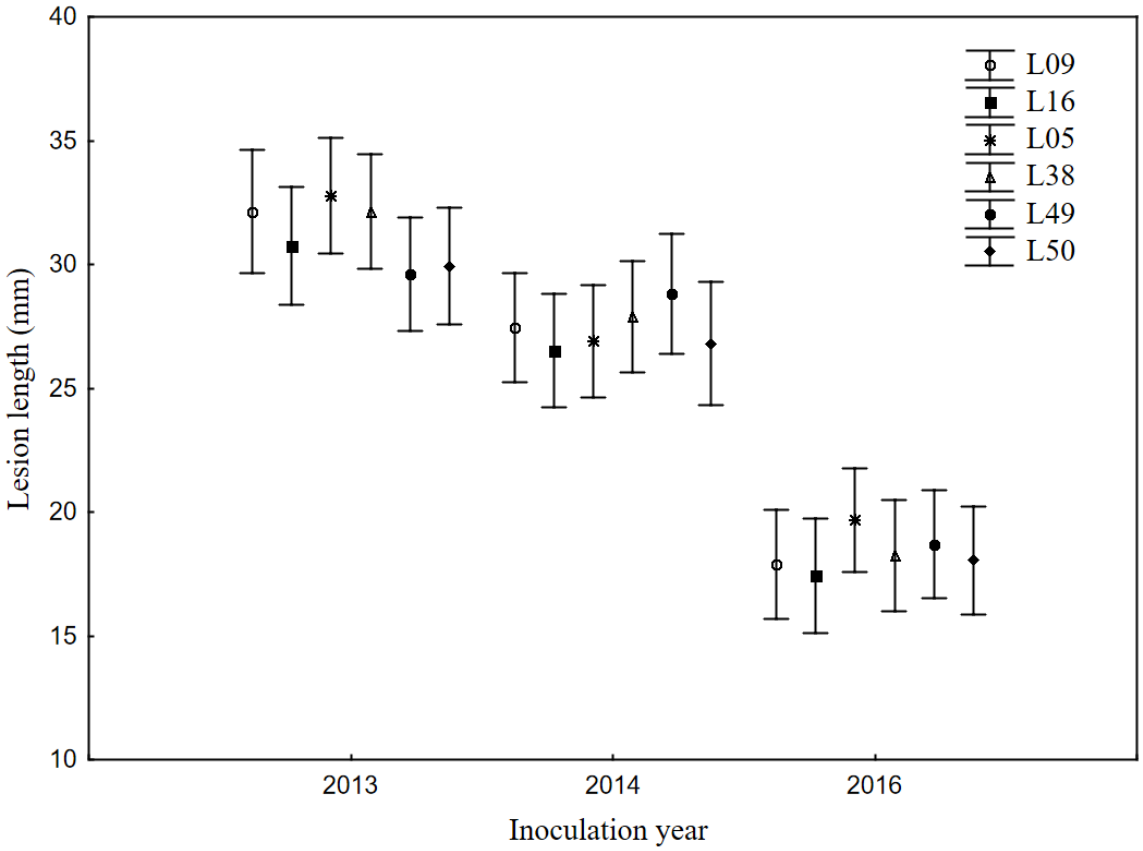


**Figure 1.17** Mean lesion length of connector *Pinus taeda* families on each inoculation year. Error bars represent 95% confidence interval.

**Table 1.1** *Pinus taeda* families for the year 2013.

| Family ID | Company |
|---|---|
| L05 | Rayonier |
| L09 | Rayonier |
| L16 | Rayonier |
| L38 | Arborgen |
| L49 | Arborgen |
| L50 | Arborgen |
| L51 | Rayonier |
| L52 | Arborgen |
| L53 | Westervelt |
| L54 | Rayonier |
| L55 | Rayonier |
| L56 | Rayonier |
| L57 | Rayonier |
| L58 | Weyerhaeuser |
| L59 | Weyerhaeuser |
| L60 | Weyerhaeuser |
| L61 | Weyerhaeuser |
| L62 | Plum Creek |
| L63 | Plum Creek |
| L64 | Plum Creek |
| L65 | Plum Creek |
| L66 | Arborgen |
| L67 | Arborgen |
| L68 | Arborgen |
| L69 | Hancock |
| L70 | Hancock |
| L71 | Hancock |
| L72 | Hancock |
| L73 | Hancock |
| L74 | Westervelt |
| L75 | Westervelt |
| L76 | Westervelt |
| L77 | Westervelt |

Notes: L05, L09, L16, L38, L49 and L50 replicated each year, L49: FM2 and L50: SEF-Mix are wildtype families

**Table 1.2** *Pinus taeda* families for the year 2014.

| Family code | Company |
|---|---|
| L78 | Arborgen |
| L79 | Weyehaeuser |
| L80 | Rayonier |
| L81* | Arborgen |
| L16 | Rayonier |
| L82 | Arborgen |
| L83 | Weyehaeuser |
| L84 | Westervelt |
| L85 | Arborgen |
| L86 | Plum Creek |
| L05 | Rayonier |
| L87 | Arborgen |
| L88 | Plum Creek |
| L38* | Arborgen |
| L89 | Plum Creek |
| L90 | Westervelt |
| L91 | Plum Creek |
| L92 | Arborgen |
| L93 | Arborgen |
| L94 | Westervelt |
| L95 | Westervelt |
| L09* | Rayonier |
| L96 | Hancock |
| L97 | Hancock |
| L98 | Arborgen |
| L99 | Rayonier |
| L100 | Rayonier |
| L101 | Rayonier |
| L102 | Weyehaeuser |
| L103 | Westervelt |
| L104 | Arborgen |
| L105 | Arborgen |
| L106 | Weyehaeuser |
| L107 | Hancock |
| L49 | Arborgen |
| L108 | Weyehaeuser |
| L109* | Plum Creek |
| L50 | Arborgen |

Notes: L05, L09, L16, L38, L49 and L50 replicated each year, L49: FM2, L50: SEF-Mix are wildtype families, * represents families with both bare-root and container-grown seedlings.

**Table 1.3** *Pinus taeda* families for the year 2016.

| Family code | Company |
|---|---|
| L114 | Rayonier |
| L118 | Rayonier |
| L05 | Rayonier |
| L16 | Rayonier |
| L123 | Rayonier |
| L117 | Rayonier |
| L124 | Rayonier |
| L116 | Plum Creek |
| L122 | Plum Creek |
| L115 | Plum Creek |
| L111 | Westervelt |
| L33 | Westervelt |
| L09 | Weyehauser |
| L126 | Weyehauser |
| L127 | Weyehauser |
| L128 | Weyehauser |
| L129 | Weyehauser |
| L130 | Weyehauser |
| L112 | Plum Creek |
| L113 | Plum Creek |
| L38 | Arborgen |
| L49 | Arborgen |
| L50 | Arborgen |

Notes: L05, L09, L16, L38, L49 and L50 replicated each year, L49: FM2, L50: SEF-Mix are wildtype families.

**Table 1.4** Covariance parameter estimates form mixed-model (Year 2013).

| Variable | Cov Parm | Estimate | SE | Z Value | Pr > Z |
|---|---|---|---|---|---|
| Lesion length | Family | 2.88 | 1.42 | 2.03 | 0.0267 |
| | Family*Trt | 1.74 | 1.16 | 1.50 | 0.0710 |
| | Residual | 116.24 | 3.26 | 35.70 | <0.0001 |
| Lesion width | Family | 0.03 | 0.03 | 0.88 | 0.1901 |
| | Family*Trt | 0.01 | 0.04 | 0.31 | 0.3248 |
| | Residual | 6.53 | 0.18 | 35.69 | <0.0001 |
| Occlusion length | Family | 5.80 | 15.94 | 0.36 | 0.3546 |
| | Family*Trt | 28.33 | 20.41 | 1.39 | 0.0832 |
| | Residual | 915.17 | 39.02 | 23.45 | <0.0001 |
| Occlusion width | Family | 0.01 | 0.16 | 0.08 | 0.4761 |
| | Family*Trt | 0.14 | 0.21 | 0.65 | 0.2652 |
| | Residual | 10.33 | 0.46 | 22.37 | <0.0001 |

Note: Trt: Fungal treatment.

**Table 1.5** Type three fixed effects from mixed-model. Initial root collar diameter (RCD) was used as covariate (year 2013).

| Variable | Effect | DF | F Value | Pr>F |
|---|---|---|---|---|
| Lesion length | RCD | 1 | 1.68 | 0.2000 |
| | Block | 5 | 44.74 | <0.0001 |
| | Trt | 1 | 369.20 | <0.0001 |
| | Block*Trt | 5 | 33.74 | <0.0001 |
| Lesion width | RCD | 1 | 29.10 | <0.0001 |
| | Block | 5 | 22.08 | <0.0001 |
| | Trt | 1 | 323.42 | <0.0001 |
| | Block*Trt | 5 | 17.57 | <0.0001 |
| Lesion depth | RCD | 1 | 9.53 | <0.0020 |
| | Block | 5 | 4.43 | <0.0005 |
| | Trt | 1 | 17.83 | <0.0001 |
| | Block*Trt | 5 | 0.31 | <0.9000 |
| Occlusion length | RCD | 1 | 0.35 | <0.5558 |
| | Block | 5 | 20.91 | <0.0001 |
| | Trt | 1 | 16.99 | <0.0001 |
| | Block*Trt | 5 | 9.40 | <0.0001 |
| Occlusion width | RCD | 1 | 3.04 | 0.0800 |
| | Block | 5 | 10.71 | <0.0001 |
| | Trt | 1 | 4.75 | 0.0300 |
| | Block*Trt | 5 | 2.58 | 0.0200 |
| Occlusion depth | RCD | 1 | 67.86 | <0.0005 |
| | Block | 5 | 4.99 | 0.0030 |
| | Trt | 1 | 62.25 | <0.0001 |
| | Block*Trt | 5 | 1.85 | 0.1300 |

Note: RCD: Root-collar diameter and Trt: Fungal treatment.

**Table 1.6** Pairwise comparisons between all inoculation treatments for lesion length.

| Treatment | Estimate (mm) | Standard error | Adj P |
|---|---|---|---|
| GH vs LT | 7.10 | 0.50 | <0.0001 |
| GH vs W | 14.89 | 0.50 | <0.0001 |
| GH vs WM | 14.92 | 0.50 | <0.0001 |
| LT vs W | 7.78 | 0.50 | <0.0001 |
| LT vs WM | 7.82 | 0.50 | <0.0001 |
| W vs WM | 0.04 | 0.50 | 0.9999 |

Note: GH: *Grosmannia huntii*, LT: *Leptographium terebrantis*, W: Wound, WM: Wound + media.

**Table 1.7** Pairwise comparisons between all inoculation treatments for occlusion length.

| Treatment | Estimate (mm) | Standard error | Adj P |
|---|---|---|---|
| GH vs LT | 13.26 | 0.67 | <0.0001 |
| GH vs W | 29.19 | 0.67 | <0.0001 |
| GH vs WM | 28.02 | 0.67 | <0.0001 |
| LT vs W | 15.94 | 0.67 | <0.0001 |
| LT vs WM | 14.76 | 0.67 | <0.0001 |
| W vs WM | -1.18 | 0.67 | 0.2933 |

Note: GH: *Grosmannia huntii*, LT: *Leptographium terebrantis*, W: Wound, WM: Wound + media.

**Table 1.8** Covariance parameter estimates from the mixed model (Year 2014).

| Parameter | Covariance Parameter | Estimate | Standard Error | Z values | Pr>Z |
|---|---|---|---|---|---|
| Lesion length | Family | 6.16 | 2.16 | 2.85 | 0.0022 |
| | Family*treatment | 1.46 | 1.26 | 1.16 | 0.1230 |
| | Residual | 149.07 | 3.94 | 37.85 | <0.0001 |
| Occlusion length | Family | 7.34 | 3.49 | 2.13 | 0.0166 |
| | Family*treatment | 4.60 | 2.89 | 1.59 | 0.0620 |
| | Residual | 289.50 | 7.69 | 37.65 | <0.0001 |

**Table 1.9** Type three fixed effects from the mixed model (Year 2014).

| Variables | Effect | Num DF | Den DF | F value | Pr>F |
|---|---|---|---|---|---|
| Lesion length | RCD | 1 | 2864 | 6.98 | 0.0083 |
| | Block | 5 | 2864 | 117.11 | <0.0001 |
| | Trt | 1 | 2864 | 179.62 | <0.0001 |
| | Block*Trt | 5 | 2864 | 115.03 | <0.0001 |
| Lesion width | RCD | 1 | 2864 | 137.60 | <0.0001 |
| | Block | 5 | 2864 | 74.62 | <0.0001 |
| | Trt | 1 | 2864 | 75.75 | <0.0001 |
| | Block*Trt | 5 | 2864 | 10.29 | <0.0001 |
| Lesion depth | RCD | 1 | 2864 | 68.03 | <0.2426 |
| | Block | 5 | 2864 | 16.98 | <0.0001 |
| | Trt | 1 | 2864 | 35.98 | <0.0001 |
| | Block*Trt | 5 | 2864 | 53.15 | <0.0001 |
| Occlusion length | RCD | 1 | 2833 | 13.02 | 0.0003 |
| | Block | 5 | 2833 | 187.50 | <0.0001 |
| | Trt | 3 | 2833 | 295.73 | <0.0001 |
| | Block*Trt | 15 | 2833 | 245.55 | <0.0001 |
| Occlusion width | RCD | 1 | 2833 | 266.42 | <0.0001 |
| | Block | 5 | 2833 | 234.15 | <0.0001 |
| | Trt | 3 | 2833 | 574.56 | <0.0001 |
| | Block*Trt | 15 | 2833 | 174.19 | <0.0001 |
| Occlusion depth | RCD | 1 | 2832 | 177.16 | <0.0001 |
| | Block | 5 | 2832 | 147.25 | <0.0001 |
| | Trt | 3 | 2832 | 427.68 | <0.0001 |
| | Block*Trt | 15 | 2832 | 161.18 | <0.0001 |

Note: Trt: Treatment, RCD: Root-collar diameter.

**Table 1.10** Pairwise comparison of log lesion between the treatments.

| Treatment | Estimate | Standard error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| GH vs LT | -0.01 | 0.01 | 3943 | -10.39 | <0.0001 |
| GH vs W | 0.40 | 0.01 | 3943 | 40.60 | <0.0001 |
| GH vs WM | 0.31 | 0.01 | 3943 | 32.56 | <0.0001 |
| LT vs W | 0.50 | 0.01 | 3943 | 51.74 | <0.0001 |
| LT vs WM | 0.41 | 0.01 | 3943 | 43.75 | <0.0001 |
| W vs WM | -0.09 | 0.01 | 3943 | -8.99 | <0.0001 |

Note: GH: *Grosmannia huntii*, LT: *Leptographium terebrantis*, W: Wound, and WM: Wound + media.

**Table 1.11** Treatment and family fixed effects from mixed model.

| Variable | Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|---|
| Lesion length | Block | 5 | 1971 | 178.45 | <0.0001 |
| | IRCD | 1 | 1971 | 8.88 | 0.0029 |
| | Family | 22 | 1971 | 4.48 | <0.0001 |
| | Trt | 1 | 1971 | 137.51 | <0.0001 |
| | Trt*Family | 22 | 1971 | 2.10 | 0.002 |
| Lesion width | Block | 5 | 1963 | 26.06 | <0.0001 |
| | IRCD | 1 | 1963 | 13.23 | 0.0003 |
| | Family | 22 | 1963 | 2.54 | 0.0001 |
| | Trt | 1 | 1963 | 206.43 | <0.0001 |
| | Trt*Family | 22 | 1963 | 1.07 | 0.3784 |
| Lesion depth | Block | 5 | 1971 | 69.23 | <0.0001 |
| | IRCD | 1 | 1971 | 39.99 | <0.0001 |
| | Family | 22 | 1971 | 2.15 | 0.0015 |
| | Trt | 1 | 1971 | 130.79 | <0.0001 |
| | Trt*Family | 22 | 1971 | 1.13 | 0.3049 |
| Occlusion length | Block | 5 | 1952 | 223.98 | <0.0001 |
| | IRCD | 1 | 1952 | 5.49 | 0.0193 |
| | Family | 22 | 1952 | 5.16 | <0.0001 |
| | Trt | 1 | 1952 | 552.47 | <0.0001 |
| | Trt*Family | 22 | 1952 | 2.65 | <0.0001 |
| Occlusion depth | Block | 5 | 1952 | 36.32 | <0.0001 |
| | IRCD | 1 | 1952 | 37.41 | <0.0001 |
| | Family | 22 | 1952 | 2.51 | 0.0001 |
| | Trt | 1 | 1952 | 587.09 | <0.0001 |
| | Trt*Family | 22 | 1952 | 1.75 | 0.0171 |
| Occlusion width | Block | 5 | 1952 | 112.38 | <0.0001 |
| | IRCD | 1 | 1952 | 29.71 | <0.0001 |
| | Family | 22 | 1952 | 2.93 | <0.0001 |
| | Trt | 1 | 1952 | 546.88 | <0.0001 |
| | Trt*Family | 22 | 1952 | 1.55 | 0.0505 |

Note: RCD: Initial Root-collar-diameter, Trt: Treatment.