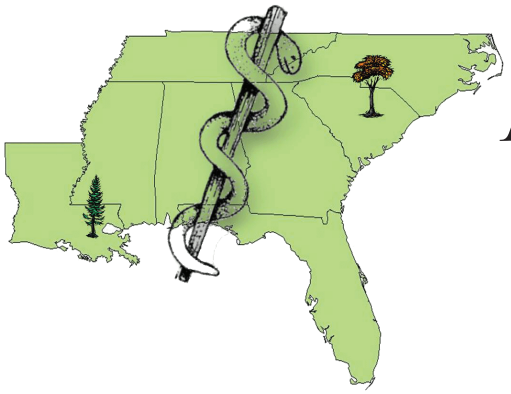

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THE ROLE OF *LEPTOGRAPHIUM TEREBRANTIS* AND *GROSMANNIA HUNTII* INVASION IN DRIVING DROUGHT-RELATED DECLINE IN *PINUS TAEDA* FAMILIES

by
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ABSTRACT
The complex interaction of various biotic and abiotic factors may put the overall stand health of *Pinus* spp. at risk. A study was designed to determine the combined impact of drought and vascular-inhabiting fungi (*Leptographium terebrantis* and *Grosmannia huntii*) in *Pinus taeda* (loblolly pine). Seedlings from two *P. taeda* families were planted and watering treatments: i. normal moisture, ii. medium drought, and iii. severe drought were applied. One month following initiation of watering treatments, seedling stems were artificially inoculated with *L. terebrantis* and *G. huntii*. Drought and fungal interaction significantly affected lesion and occlusion length, and seedling fine root dry matter biomass yield and needle-to-fine-root dry biomass ratio. Leptographium terebrantis was more virulent in severe drought conditions. The tolerant family was also more tolerant to drought, indicating tolerance to of *P. taeda* to drought may be improved through appropriate family selection. Drought and vascular-inhabiting fungi may negatively impact *P. taeda* stand health.

INTRODUCTION
Adverse climatic conditions like drought have been shown to be responsible for a number of forest declines throughout the world (Adams et al., 2013; Cailleret et al., 2014). Recent incidents of tree decline and mortality have been related to increased mean annual temperatures and decreased mean annual rainfall in European forests (Carnicer et al., 2011) and increased droughts in the southwestern (van Mantgem et al., 2009) and southeastern U.S. (Klos et al., 2009; Wang et al., 2010). Drought events are expected to become more common in future (IPCC, 2013) resulting in drought-induced forest mortality (Peng et al., 2011). As forests play a key role in the global water and carbon cycles, a feedback loop exists between climate change and forest function (Bonan, 2008). Despite the effects of drought on forest function, mechanisms underlying forest decline and mortality are still weakly understood (Mc Dowell et al., 2011).

According to Turtola et al. (2003), low soil moisture influences the production of specific chemicals in conifers rendering the trees more susceptible to both pathogen and insect attack. For example, bark beetle infestation in drought-weakened *Pinus* forests may occur many years after the end of the climatological drought (Raffa et al., 2008). Beetle-vectored, vascular-inhabiting pathogens can have a devastating effect on drought stressed trees (Oliva et al., 2014). Vascular wilt pathogens such as *Ceratocystis* Ellis and Halst., *Leptographium*, and *Grosmannia* Goid. species thrive in the xylem of *Pinus* spp. (Yadeta and Thomma, 2013; Singh et al., 2014). Pinus spp. defend against these fungi by producing resins that clog the plant vascular conducting tissues (Matusick and Eckhardt, 2010). Clogging of plant conduits disturb plant water transport, resulting in hydraulic

failure that leads to tree mortality (Oliva et al., 2014). Thus, adverse climatic conditions easily influence susceptibility of conifer hosts to pathogen and insect attack (Lindberg and Johansson, 1992).

Models of forest decline incorporate predisposing factors, inciting factor and contributing factors (Sinclair 1966; Manion, 1981). In this context, *P. taeda* L. decline in the southern U.S. has been associated with (i) predisposing factors like tree genetics, age, ozone and adverse soil conditions, (ii) inciting factors such as drought and increased ozone and (iii) contributing factors like root-feeding bark beetles and beetle-vectored vascular-inhabiting fungi (Eckhardt et al., 2004; Eckhardt and Menard, 2008; Eckhardt et al., 2010). The vascular inhabiting fungal pathogens are considered to be the major driving factors in the final phase of drought-induced tree and stand mortality (Oliva et al., 2014).

Pathologists many have been suffering from false dichotomy of drought vs biotic attack (Mc Dowell et al., 2013). Many studies have focused primarily on individual factors: (i) drought and its subsequent effect on plant physiology (Noormets et al., 2010; Maggard et al., 2016) or (ii) biotic agents and its subsequent impact on tree health (Matusick and Eckhardt, 2010; Singh et al., 2014). However, the evidence for the mechanisms suggested by these individual factors is inconclusive and a more integrated approach focusing on interrelations between drought and the biotic agents on tree growth and functioning are needed.

Recently, a limited number of studies have concentrated on the interaction of drought and vascular-inhabiting fungi (Matusick et al., 2008; Chieppa et al., 2017). However, these studies were conducted for shorter time periods and deployed both drought and fungal treatment at the same time (Matusick et al., 2008; Chieppa et al., 2017), despite the fact that these vascular-inhabiting fungi come into play only after the predisposition of trees to a drought event. Thus, a closer examination of the impact of *L. terebrantis* and *G. huntii* on *P. taeda* trees predisposed to drought is needed. In this concern, we address the following questions: (i) Does the virulence of *L. terebrantis* and *G. huntii* in *P. taeda* increase under increasing drought?, (ii) Does drought stress increase the susceptibility of *P. taeda* families to these fungi?, and (iii) Is infection by vascular-inhabiting fungi under drought likely to enhance tree decline directly through increased investment in occlusion and indirectly through a reduction of plant growth?

METHODOLOGY

Experimental set-up

The experiment was conducted in the research facility of the Southern Forest Nursery Management Cooperative Auburn, AL, USA. The facility contained an open outdoor pavilion with 12 raised wooden boxes (120 cm long and 100 cm wide) filled with pure sand. Plastic transparent roof covered the pavilion to exclude ambient rainfall. A system was used for automatic irrigation prior to the commencement of the study.

Seedling planting

One-year-old, bare-root seedlings from two commercially grown *P. taeda* families were used. Seeds were sown in March 2014 and seedlings were lifted from the nursery in January 2015. Based

on previous findings by Singh et al., (2014), one family was considered "susceptible" (S) and one "tolerant" (T) to root-infecting ophiostomatoid fungi. In February 2015, 630 seedlings (35 per family in each box) were planted in 9 wooden boxes and watered to field capacity for 4 weeks until watering treatments were initiated.

Moisture treatment

Three watering treatments: i. normal moisture, ii. moderate drought, and iii. severe drought were deployed to 3 boxes (3 replicates/treatment) in March 2015. The watering treatments were determined based on the volumetric water content of the pure sand and loblolly pine. The well-watered soil sample was taken with soil core of known volume. The wet weight and dry weight (72 h at 105 ºC) of the soil were determined and the volumetric water content of the soil sample at field capacity (FC) was determined by using the following formula:

$$V = \frac{Wmass - Dmass}{Pw\,Vs}$$

Where *Wmass* is the mass before drying and *Dmass* is the mass after drying, *Pw* is the density of water (1000 kg m^{-3}) and *Vs* is the total volume of the soil sample (sum of air, water, and soil). The volumetric water content for the FC was 32 $m^3\,m^{-3}$. The watering treatments were as follows: i. 75 % of FC (normal water i.e 28 $m^3\,m^{-3}$), ii. 50 % of FC (medium drought i.e. 18 $m^3\,m^{-3}$), and iii. 25 % of FC (severe drought i.e. 11 $m^3\,m^{-3}$). Soil water content was constantly monitored in each box using an external moisture probe (Figure 1.2) and irrigation was programmed to meet volumetric water content of each box.

Inoculation treatment

One month into the three watering treatments (April 2015), artificial stem inoculations were conducted using the method described by Nevill et al. (1995), Singh et al. (2014), and Chieppa et al. (2017) using wound + inoculum method. Five inoculation treatments applied were as follows: *L. terebrantis* (LOB-R-00-805/ATCC accession no. MYA-3316), *G. huntii* (LLP-R-02/ATCC accession no. MYA-3311), wound, wound + media and no wound. Seven seedlings per family within a box received each inoculation treatment. The *L. terebrantis* isolate used was isolated from a root of *P. taeda* exhibiting local tissue damage and deteriorating crowns from the Talladega National Forest, Oakmulgee Ranger District, AL, USA (Eckhardt et al., 2007). The *G. huntii* isolate used was isolated from a root of *P. palustris* Mill. showing similar characteristics of decline from the Fort Benning Military Reservation, GA, USA. These isolates have been used in previous artificial inoculation studies (Matusick and Eckhardt, 2010; Singh et al., 2014; Chieppa et al., 2017). The fungal isolates were maintained at 4 ºC in Malt Extract Agar (MEA) before use and were plated on 2 % MEA plate, 14 days prior to the inoculation experiment.

To perform the inoculation, 13 mm (<2 mm depth) of seedling bark at the stem, ~ 3 cm above soil line was cut vertically with a sterile razor blade. The single pre-punched plug of agar (3 mm) with actively growing fungal mycelium was placed (fungus-side-towards wound) in the wound in each seedling. Sterile agar was inoculated in the wound in case of wound + media inoculation. A sterile cut was made for wound control. No wound was made in seedling receiving no wound treatment. Wounds on the stems were then wrapped with sterile cotton balls moistened with deionized water to prevent desiccation of MEA and wrapped with Parafilm® to avoid contamination.

Pre-harvesting measurements

Growth and size measurement

Height and root-collar diameter (RCD) measurements were collected from each seedling prior to watering treatment (March 2015), stem inoculation (April 2015) and seedling harvesting (September 2015). Before seedling planting (February 2015), 12 extra seedlings from each family were randomly selected for height, RCD, and biomass measurements and the averages are given in Table 1.1. The number of new buds developed were also counted on individual seedlings before watering (March 2015) and inoculation treatments (April 2015) and prior to seedling harvesting (September 2015).

Needle greenness and chlorophyll content

Needle greenness was measured non-destructively on 5-7 needles that reached physiological maturity using Soil-Plant Analysis Development-502 (SPAD) chlorophyll meter (Spectrum Tech. Inc., Plainfield, IL, USA) prior to inoculation treatment and at seedling harvest. Three measurements were taken from each seedling and then averaged.

Needle chlorophyll content was measured in two needles for all treatments. Samples were collected by extracting 0.25 g of the needle material in 6.25 ml 95 % ethanol. The sample was kept in the dark in a water bath for 24 h and then allowed to cool to room temperature and vortexed at slow speed for 1 minute. Samples were centrifuged for 5 minutes at 13500 g, and the extract was collected. The extract absorbance was measured at 665, 645 and 470 nm using a UV-Vis spectrophotometer (Thermo ScientificTM, USA). Chlorophyll content Chlorophyll-a (Chla) and Chlorophyll-b (Chlb) was calculated by using the following formula (Guo et al., 2010):

$$Chla = 13.95A665 - 6.88A649$$

and

$$Chlb = 24.96A649 - 7.32A665$$

Predawn water potential

Two seedlings from each treatment, replicate, were destructively sampled (end of August 2015) for pre-dawn needle water potential (Ψpd) measurements. Three mature fascicles from each seedling were harvested between 2:30 AM and 5:00 AM, placed in an air-tight plastic bag and kept in a dark box until measured. A Scholander pressure bomb (PMS Instrument Company, Albany, OR, USA) was used to measure Ψpd, within 5 hours of collection.

Relative leaf water content

The relative leaf water content (RWC) was measured on needle fascicles used for the water potential measurement. The fresh weight of the fascicle was determined then soaked in sterile deionized water for 24 h. The fascicle was oven dried at 75 ºC for 48 h, re-weighed and RCW was calculated by the following formula:

$$\boldsymbol{RCW = \frac{W-DW}{TW-DW}\ X\ 100\ \%}$$

Where *W* = Sample fresh weight, *TW* = Turgid weight of needle, *DW* = Dry weight of the sample.

Post-harvest measurement

Inoculation response

In September 2015, four seedlings from each treatment within each box were cut at the stem above the soil level (September 2015). Seedling stems were placed in mixture of stain (FastGreen FCF; Sigma Chemical Co., St. Louis, MO, USA) and water in a ratio of 0.25 g L^{-1} for 72 h. The bark near the inoculation point was carefully scraped away to the xylem and the lesion and occlusion length and width were measured. The phloem colonized by the fungi was measured as the lesion. The portion of the xylem that did not allow the stain to pass through it was considered an occlusion. Two pieces (~ 3 mm) of stem tissue surrounding the lesion were cut and plated on MEA with cycloheximide at 800 mg L^{-1} and streptomycin sulfate at 200 mg L^{-1} to confirm fungal re-isolation. Stem sections of control seedlings also were also plated to confirm no contamination.

Seedling biomass

Three remaining seedlings from each treatment combination per box were used for dry biomass measurements. Each seedling was separated into needles (N), stem (S), coarse root (CR) and fine root (FR) and allowed to dry at 75 ºC for 72 h then weighed.

Statistical analysis

The experimental design was a randomized complete block (RCBD) design with replicates at all levels. The data for the seedling survival were analyzed by logistic regression using R 3.3.1.
Similarly, the general linear model was used to analyze the other response variables. The general linear model used was:

$$y = \beta o + \beta 1 + \beta 2 + \beta 3 + \beta 4 + \beta 5 + \beta 6 + \beta 7 + \mathcal{E}$$

Where, y is the response variable (lesion and occlusion length, depth and width, and needle, stem, coarse root, fine root). $\beta 0$ is the intercept, $\beta 1$ is the family effect, $\beta 2$ is the fungal effect, $\beta 3$ is the moisture effect, $\beta 4$ is the family x fungal interaction, $\beta 5$ is the interaction of fungal treatment x moisture treatment and $\beta 6$ is the interaction of family x moisture treatment and $\beta 7$ is the random effect of the box and $\mathcal{E}$ is the residual error. Multiple comparisons tests were performed by using post hoc Tukey (Honest Significant Difference) procedures. All the assumptions of normality and homogeneity of the variance were inspected. All the statistical analysis were conducted using SAS (Version 9.4, SAS Institute, Inc., Cary, NC, USA), R Core 3.3.1 and STATISTICA (Statsoft, Inc., Tulsa, OK, USA).

RESULTS

Seedling survival

No specific pattern regarding the seedling mortality was observed during the study period. Seedling survival was not affected by family of *P. taeda*, watering treatment, and fungal inoculation. An overall result of the effects of different treatments in the response variables is presented in Table 1.2.

Lesion and occlusion

Dark brown necrotic tissues were observed at the inoculation point in all the inoculated seedlings. The re-isolation of *G. huntii* and *L. terebrantis* was 89% and 92%, respectively indicating successful fungal inoculation. Seedling lesions with the control inoculations were significantly smaller than fungal inoculations, indicating fungi, but not the wound caused lesion. The lesions in the wound and wound + media did not extend beyond the inoculation zone.

Lesion length/seedling height (LL/Ht) was significantly affected by family and the interaction between inoculation and watering conditions (Table 1.2). Family S (susceptible family) had the greatest lesion and lesion length/height ratio. *L. terebrantis* resulted in the highest lesion length/height ratio in severe drought condition (Figure 1.6).

Lesion length was significantly affected by watering treatment, family, inoculation, family x inoculation and watering treatment x inoculation. *Leptographium terebrantis* resulted in significantly longer lesion than *G. huntii* within both tolerant (T) and susceptible (S) family. The seedlings under severe drought challenged with *L. terebrantis* had the longest lesions (Table 1.3). Under the medium drought condition, there was greater variation in lesion length caused by both fungi. The lesion caused by *L. terebrantis* was significantly longer in the susceptible family than the tolerant family.

Lesion depth was significantly affected by the watering condition, inoculation and family x watering x inoculation (Table 1.2). *Leptographium terebrantis* caused deeper lesions than *G. huntii* in all treatment and treatment combinations (Table 1.5). Occlusion length was significantly affected by family, inoculation, family x inoculation, watering treatment x inoculation interaction (Table 1.2). The occlusion length caused by the wound and wound + media inoculation was not different among the various watering treatments and smaller than the caused by fungal inoculation. *Leptographium terebrantis* caused the longest length of occlusion in seedlings under severe drought (Table 1.3).

Occlusion length/seedling height was affected by watering treatment, family, inoculation and watering treatment x inoculation interaction (Table 1.2). Within each watering treatment, *L. terebrantis* caused significantly higher occlusion length/seedling height than *G. huntii* (Table 1.3). Occlusion length was also affected by family, inoculation, family x inoculation, and watering treatment x inoculation (Table 1.2). *Leptographium terebrantis* caused longer occlusion lengths than *G. huntii* in both families (Table 1.4). Seedlings under moderate drought had greatest occlusion length as a response to *L. terebrantis* inoculation. However, the occlusion length/seedling height ratio was not significantly different between the seedlings receiving different watering treatments. This ratio was significantly higher in seedlings inoculated with *L. terebrantis* than *G. huntii* (Table 1.3), indicating the higher virulence of *L. terebrantis*.

Occlusion depth produced in the seedlings was significantly affected by watering treatment, family type, and inoculation. *Grosmannia huntii* and *L. terebrantis* caused significantly deeper occlusions than wound and wound + media inoculated seedlings, suggesting successful fungal inoculation. *Leptographium terebrantis* caused deeper occlusion than *G. huntii* under all watering conditions.

Seedling volume increment

Watering treatment, family, inoculation, and family x inoculation interaction significantly affected seedling growth during the experiment (Table 1.2). The susceptible family (S) grew less than tolerant family (T). This reduction in growth rate cannot be attributed to the response of different family to fungal inoculation as the growth was not significantly different between the fungi inoculated and control seedlings. Thus, the tolerant family has more capability to grow compared to susceptible family. Volume increment of seedlings under drought stress was low.

Bud-outbreak number increment

Bud-outbreak was significantly affected by inoculation x watering treatment. The bud production was not affected by family. The control (NW) had the greatest number of the buds with no differences among medium drought and normal watering. Seedlings challenged with *G. huntii* had significantly fewer buds in the seedling under the severe drought than that under the high and medium drought. However, the increment in the number of bud-outbreak was not different in the seedlings inoculated with *L. terebrantis* under different watering treatments.

Seedling height increment

Overall seedling growth was affected by the watering treatment, family type, and inoculation. Tolerant family (T) had more growth than the susceptible family (S) in all 3 of the watering conditions (Table 1.10). The growth increase was significantly less in seedlings inoculated with *G. huntii* than control seedlings (Table 1.8). However, *L. terebrantis* did not affect seedling growth.

Seedling dry matter yield

Needle dry matter yield (Ny) was affected by watering treatments, family and inoculation (Table 1.2). Stem dry matter (Sy) was also significantly affected by watering treatments, family and inoculation (Table 1.2).

Watering treatments, family, and family x moisture x watering treatment significantly affected coarse root dry matter yield (Cry) (Table 1.2). *Pinus taeda* seedlings from susceptible family (S) under severe drought inoculated by *L. terebrantis* had significantly lower dry matter yield when compared to both normal watering and moderate drought. *Leptographium terebrantis* inoculation resulted in less Cry of the susceptible family under the normal watering condition. However, under both the moderate and severe drought, Cry of seedlings inoculated with *L. terebrantis* did not differ.

Fine root dry matter yield (Fry) was significantly affected by watering treatment, family, inoculation and watering treatment x inoculation. Drought treatments did not result in a significant reduction of Fry in the wound, wound + media and no wound inoculated seedlings. Under normal watering and moderate drought, *L. trerebrantis* did not reduce Fry when compared to control seedlings. However, Fry of seedlings inoculated with *L. terebrantis* was significantly less than

seedlings under severe drought as compared those under the moderate drought and normal watering condition. Fine root dry matter yield was not different between the seedlings inoculated with *L. terebrantis* and *G. huntii* under any of the watering conditions (Table 1.11).

The shoot-to-root dry matter yield ratio (Sy/Ry) was significantly different between the seedlings from two families and three moisture treatments (Table 1.2). The family considered tolerant to ophiostomatoid fungi had lower Sy/Ry ratio than family considered susceptible (Table 1.14). Seedlings under severe and moderate drought had higher Sy/Ry ratio than seedlings receiving normal watering treatment (Table 1.15). Control and fungal inoculation treatments did not affect Sy/Ry.

Similarly, the coarse-root-to fine root dry matter yield ratio (Cry/Fry) was also significantly different between seedlings from two families. The family considered tolerant to ophiostomatoid fungi had significantly higher Cry/Fry ratio than susceptible family. The *L. terebrantis* inoculated seedlings had higher Cry/Fry ratio than wound inoculated seedlings (Table 1.14).

Needle-to-fine-root dry matter yield ratio (Ny/Fry) was significantly affected by watering treatment and fungal inoculation. Seedlings inoculated with *G. huntii* and *L. terebrantis* under severe drought had significantly higher Ny/Fry ratio than that under normal watering (Table 1.16).

Needle greenness and needle chlorophyll content

The needle greenness was significantly different between the inoculation treatments ($P = <0.0001$). Seedlings treated with *L. terebrantis* and *G. huntii* had significantly lower needle greenness (Table 1.17). However, the watering treatment did not significantly impact needle greenness (Table 1.2). The needle chlorophyll content was not significantly affected by fungi ($P = 0.52671$), watering treatment, or watering treatment x inoculation interaction ($F_{(1, 35)} = 0.97$, $P = 0.43770$).

Pre-dawn water potential measurement

Pre-dawn needle water potential (Ψpd) was significantly affected by inoculation, ($P = 0.00218$) (Table 1.2). Seedlings inoculated with *L. terebrantis* and *G. huntii* had significantly more negative Ψpd than the no wound seedlings (Table 1.18). However, fungal inoculation did not cause significantly negative water potential than wound and wound + media controls.

Relative water content

The relative water content (RWC) was significantly affected by watering treatments (Table 1.2). However, it was not significantly affected by the inoculation, family type and any of the interaction. Seedling under moderate drought had significantly low needle relative water content than the normal watering condition and severe drought (Table 1.19).

DISCUSSION

The relative susceptibility of *P. taeda* families to vascular-inhabiting ophiostomatoid fungi is not influenced by different watering conditions. The tolerant family grew better than the susceptible family selected for susceptibility. *Leptographium terebrantis* was consistently more virulent as determined by lesion length to *P. taeda* than *G. huntii.* There was an interaction between watering and inoculation treatment in case of lesion length and occlusion length. Previous studies by

Matusick et al. (2008) and Chieppa et al. (2017) did not find any evidence of soil moisture and fungal interaction.

The virulence of *L. terebrantis* when inoculated into seedlings (in terms of LL/Ht, LD, and OD) increased under severe drought condition. Virulence of *G. huntii* (in terms of lesion and occlusion), however, remained same under different watering conditions. Salle et al. (2008) reported longer length by *L. yunnanense* in moderately water-stressed *P. yunnanenesis* (Franch.). We observed similar results only in terms of occlusion length. In contrast, Christiansen and Glosli (1996) reported that phloem damage and blue staining due to *Ceratocystis polonica* was greater in the well-watered trees than in the water-stressed trees. This difference in result between their study and the present study might be due to the variation in the species-specific threshold.

Virulence of *G. huntii* was increased regarding bud outbreak and Fry. Bud outbreak and Fry as a measure of seedling productivity was significantly lower in seedlings inoculated with *G. huntii* under severe drought, nevertheless, LL/Ht and OL/Ht ratio caused by the fungi did not differ between three watering conditions. This suggests that virulence of *G. huntii* increases to some extend under severe drought. Matusick et al. (2008) found no evidence in bud-break in *P. palustris* Mill. inoculated with *G. alacris* (formerly *G. serpens*) under varying soil moisture regimes at 16 weeks. This difference in result between our study and their study may be due to species-specific response.

The infection by vascular-inhabiting fungi *L. terebrantis* was likely to affect seedling health under severe drought through increased investment of seedling in LL/Ht and reduction in fine root dry yield (in both families) and coarse root dry matter yield (in the susceptible family). However, *G. huntii* inoculated seedlings under drought did not show increased defense responses to *inoculated* fungi than compared to the seedlings under the normal watering and moderate drought conditions. Also, the seedlings growth parameters were not different between the control inoculated and the fungal inoculated seedling. Thus, this may suggest that fungus *L. terebrantis* may utilize more resources of the plant under drought conditions (Oliva et al. 2014) and fungus *G. huntii* may affect the plant health independent of the drought (Chieppa et al., 2017).

Localized damage to the vascular conducting tissue was observed in inoculated *P. taeda* seedlings. The spread of the fungal mycelium into the sapwood might have caused damage to the tracheid walls. Such damage can result in cavitation and embolism (Zimmermann, 1983). The xylem blockage can be irreversible due to resin deposition and tyloses formation. Complete xylem blockage in some of the seedlings under severe drought treatment was observed. In such seedlings, surprisingly, the development of the new tissues (Figure 1.7) on the opposite side of the fungal inoculation which would have helped in the survival of the plant. However, the growth of the tissues around the fungi inoculated side was completely halted. It could be an adaptive trait of *P. taeda* that would allow the plant to be decoupled from drought as well as pathogen stress. Moreover, the growth of such seedlings was halted suggesting a potential tradeoff between this adaptive trait and plant growth.

Both the present study and few additional studies indicate that in general, the family chosen for tolerance to ophiostomatoid fungi should have more growth potential in terms of seedling volume change and height increment e.g., Chieppa et al. (2015) and Chieppa et al. (2017). *Pinus taeda*

families used in the studies above and in the current study were the same. Therefore, it is not surprising that the results between studies were similar. Inoculated and non-inoculated controls did not show any difference in growth potential suggesting wound and media do not have any effect on seedling growth. Taken together, the previous studies and present together show some support for the higher growth potential of tolerant families. Other indicators of plant growth (increment in the number of new bud-break) was not different between the two families.

The tolerant family tended to yield more stem and needle dry matter than the susceptible family. Stem and needle dry weight yield was less under severe drought conditions when compared to the normal water. To our knowledge, this is the first study to show a two-way interaction of watering x inoculation (in terms of fine root dry matter yield) and three-way interaction of watering treatment x family x inoculation (in terms of coarse root dry matter yield) in *P. taeda* under different watering treatments. Here, *L. terebrantis* caused less coarse root biomass yield in severe drought conditions. The results of our study have been supported by that of Croisé et al. (2001). Unlike, Croisé et al. (2001) we only performed single point inoculation. Massive inoculation of the fungi might lead to more detrimental effects on the *P. taeda* seedlings.

The seedlings inoculated with *L. terebrantis* under severe drought allocated significantly little biomass to roots as compared to other seedlings within same or other watering treatments. The allocation of biomass to shoot increases above and root decreases under adverse environmental conditions. Survival of plant decreases as this aboveground and belowground biomass ratio reaches a certain threshold. Above that threshold, evaporative surface (needles) increases as compared to the absorbing root surface (Cregg, 1994). Adversely, high root-to-shoot-ratio implies that the plant has the capability to cope with the drought. As the number or the length of the root increases larger soil volume can be accessed to extract more available water (Niu et al., 2008).

Inoculation of *L. terebrantis* and *G. huntii* result in a decrease in the needle greenness as compared to the control seedlings. Our results are in contrast with the previous studies performed by Chieppa et al. (2017) where they did not report any decrease in needle greenness. This discrepancy between the present study and previous study might be due to the longer study duration deployed in our study.

Inoculated *P. taeda* had lower needle water potential than the control treatments. While some seedlings inoculated with *L. terebrantis* and *G. huntii* in severe drought had water potential values lower than -1.4 MPa, no strong overall pattern was observed. The vulnerability of seedling to physiological damage may depends on individual seedling vigor. For example, it has been postulated that xylem embolism and cavitation occurs at low soil moisture levels (Croisé et al., 2001). For instance, the xylem embolism and loss of hydraulic conductivity begin at -2 MPa xylem water potential in *Pinus sylvestris* L. (Cochard, 1992). Croisé et al. (2001) reported a drop in hydraulic conductivity and needle water potential in water stressed *Pinus sylvestris* following inoculation with *L. wingfieldii*. A similar trend was not observed in the present study.

Future studies should be focused on longer-term monitoring of the fungal inoculated *P. taeda* seedlings under projected climate change scenario such as drought, increase in temperature and CO_2. The damage on an ecological scale might be higher than what we observed in our controlled study as we know that the mass attack of the beetles occurs in trees pre-stressed with drought in

the natural scenario. Thus, mass inoculation of the fungi in the stressed mature *P. taeda* trees can provide a better understanding of host-microbe and environment interactions.

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Figure 1.1 *Pinus taeda* seedlings planted in sand filled boxes experiencing different watering treatments**.**

Figure 1.2 Soil moisture being monitored with a soil moisture meter.

Figure 1.3 Inoculation of agar plug in *Pinus taeda* stem.

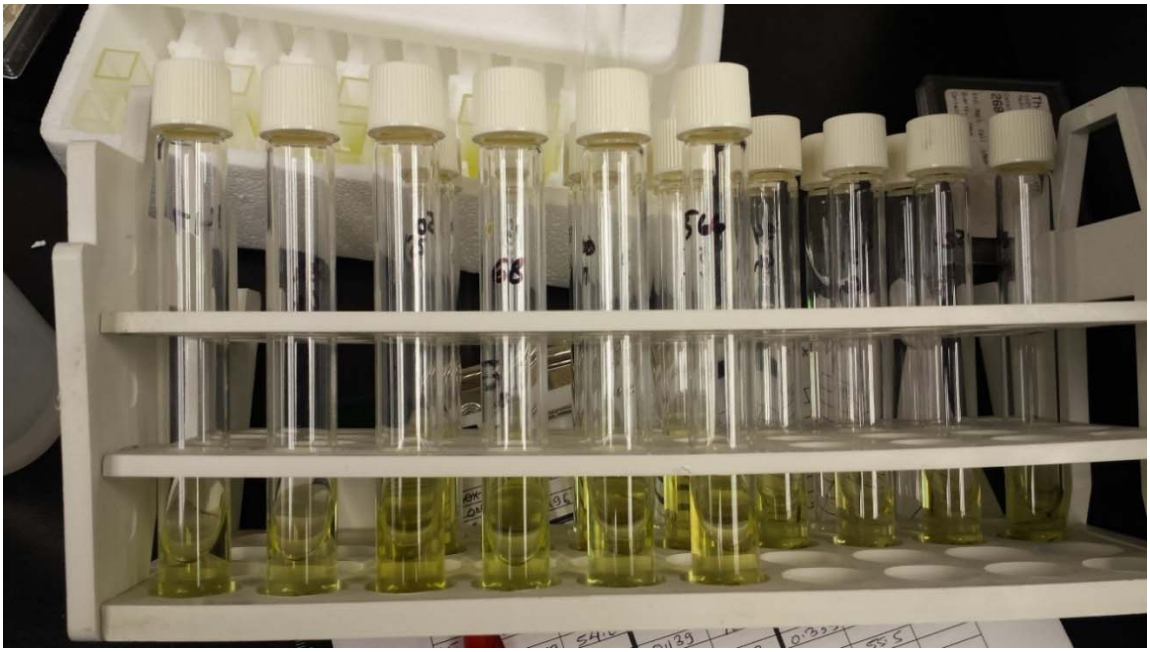

Figure 1.4 Samples containing chlorophyll extracted from *Pinus taeda* needles.

Figure 1.5 *Pinus taeda* seedling separated into coarse root (CR), fine root (FR), needles (N) and stem (S) for biomass measurement.

Figure 1.6 Necrotic tissue (lesion) on the stem of *P. taeda* seedling under severe drought inoculated with *L. terebrantis*.

Figure 1.7 Dark necrotic tissue in stem cross-section of *Pinus taeda* following fungal inoculation.

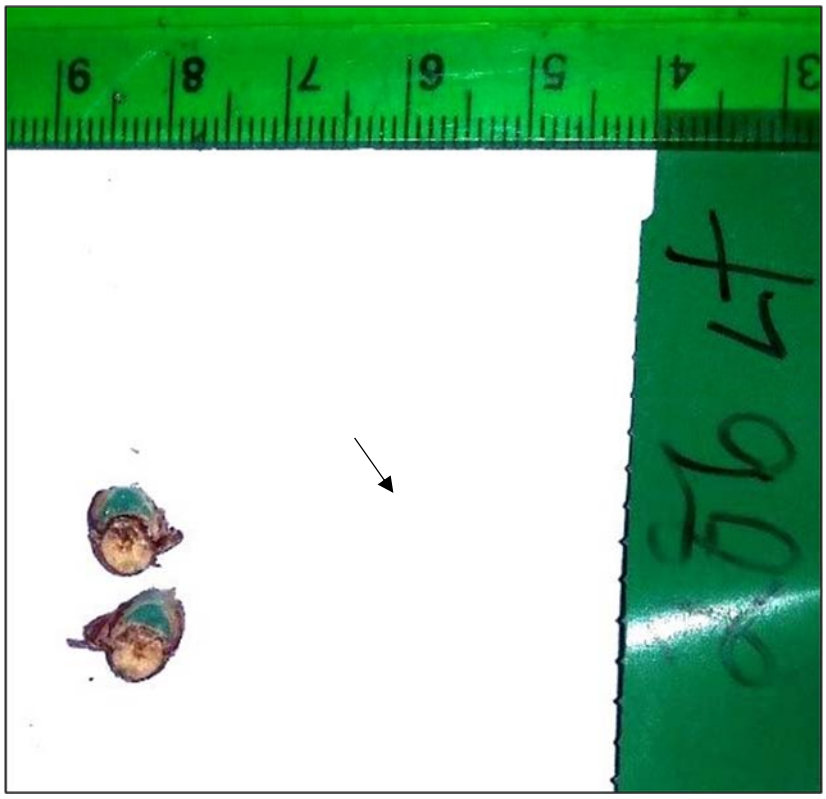

Figure 1.8 Cross-section of the *Pinus taeda* vascular tissue occluded as a result of *Leptographium terebrantis* inoculation. Arrow indicates the growth of new tissue on the opposite site of inoculation.

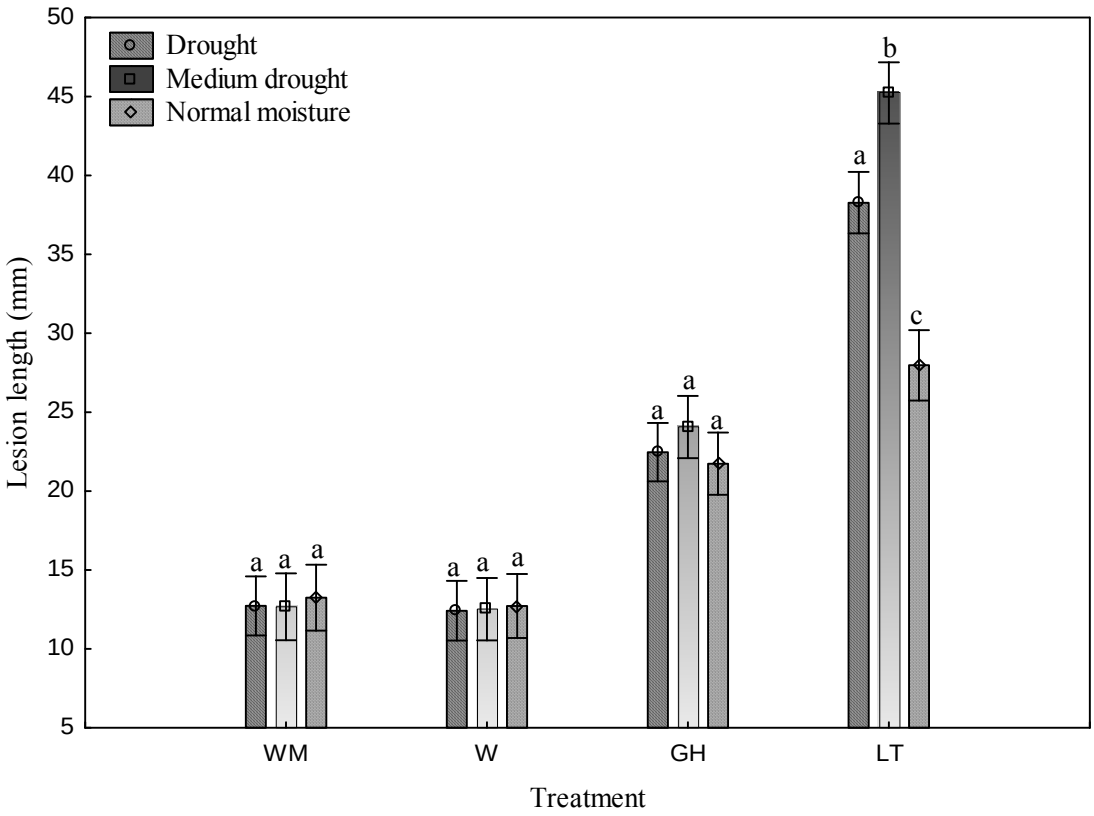

Figure 1.9 Mean lesion length produced in seedlings under different watering treatments. Different letters denote significant differences between watering treatments within each inoculation treatment at $\alpha = 0.05$.

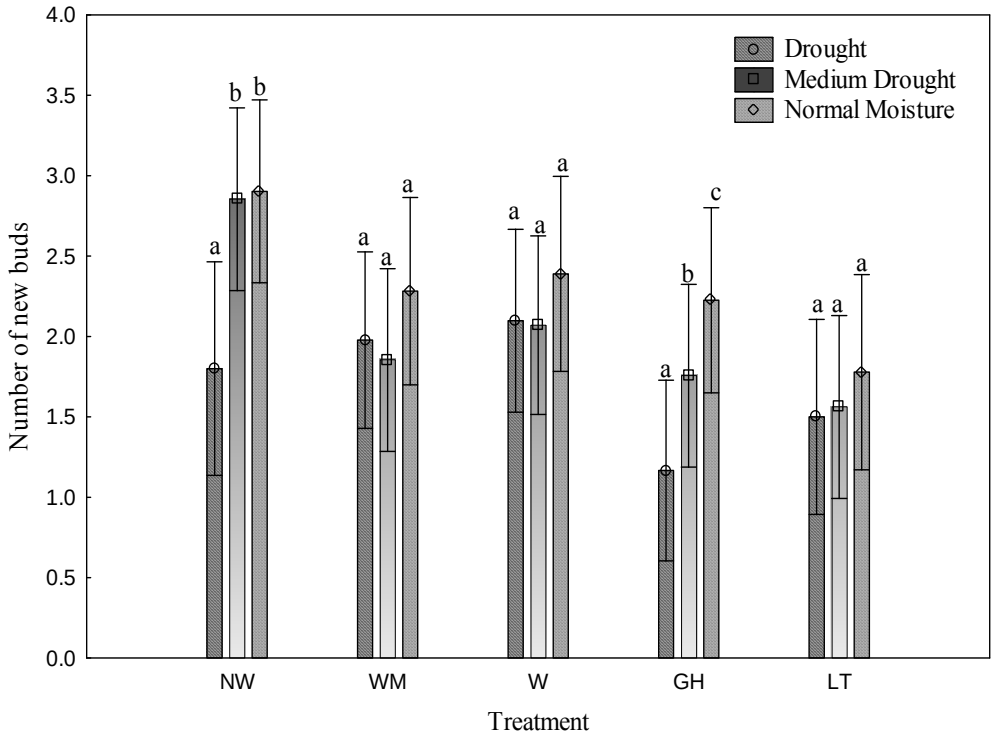

Figure 1.10 Mean numbers of bud break in seedlings under different fungal and watering treatments.

Figure 1.11 Roots of *Pinus taeda* seedling following harvest; left: Severe drought, right: Medium drought.

Figure 1.12 Seedling parts of two different *Pinus taeda* seedlings grown under different watering treatment. Left: seedling under severe drought and right: seedling under normal watering**.**

Table 1.1 Initial family height, RCD, dry wet matter of seedling averages and standard deviation (*n* = 12 per family).

F	H (cm)	RCD (mm)	FR (g)	CR (g)	N(g)	S (g)
S	23.5 (2.2)	4.29 (0.68)	0.45 (0.17)	0.39 (0.21)	1.84 (0.60)	1.10 (0.26)
T	27.5 (3.5)	5.34 (1.02)	0.89 (0.64)	0.38 (0.38)	1.48 (0.47)	1.43 (0.52)

F: Family, S and T denote loblolly pine family selected for its susceptibility and tolerance to ophiostomatoid fungi, RCD: Root-collar diameter, FR: Fine Root, CR: Coarse root, N: Needle, and S: Stem. Means followed my standard deviation in parenthesis.

Table 1.2 An overview of the effect of different treatments on seedlings response variables.

			Treatments/Combinations						
Measurement	Transformation	*n*	Mos	Fam	Ino	Mos x Fam	Fam x Ino	Mos x Ino	Fam x Mos x Ino
LL	Log10	429	***	***	***	NS	**	***	NS
LL/Ht	Log10	420	***	***	***	NS	NS	**	NS
LD	Log10	425	**	NS	***	NS	NS	NS	*
LW	Log10	419	*	NS	***	NS	NS	*	NS
OL	Log10	419	NS	*	***	NS	*	**	NS
OL/Ht	Log10	410	**	***	***	NS	NS	*	NS
OD	Log10	417	**	*	***	NS	NS	NS	NS
OW	Log10	417	**	NS	***	NS	NS	NS	NS
Ny	Log10	450	**	***	*	NS	NS	NS	NS
Sy	Log10	430	***	***	**	NS	NS	NS	NS
Cry	Log10	428	***	***	NS	NS	NS	NS	*
Fry	Raw	428	***	***	**	NS	NS	**	NS
S/R	Log10	399	***	***	NS	NS	NS	NS	NS
Fry/Cry	Log10	385	NS	*	*	NS	NS	NS	NS
Ny/Fry	Log10	375	**	NS	NS	NS	NS	**	NS
Hti	Raw	609	***	***	**	NS	NS	NS	NS
NG	Log10	315	NS	NS	***	NS	NS	NS	NS
SVC	Square root	597	***	***	**	NS	*	NS	NS
Ψpd	Log10	149	NS	NS	**	NS	NS	NS	NS
RCW	Log10	150	**	NS	NS	NS	NS	NS	NS
BP	Log10	463	*	NS	**	NS	NS	*	NS

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.0001$, Mos: watering treatment, Ino: Inoculation treatment, Fam: Family, LL: Lesion length, LL/Ht: Lesion length Height^{-1}, OL/Ht: Occlusion length Height^{-1}, LD: Lesion depth, OL: Occlusion length, Ny: Needle dry matter yield (DMY), Sy: Stem DMY, Cry: Crude root DMY, Fry: Fine root DMY, Hti: Height increase, NG: Needle greenness, SVC: Seedling volume change, *Ψpd*: Predawn water potential, RCW: Relative water content, BP: Bud production.

Table 1.3 Effects of inoculation and watering treatments on lesion and occlusion.

Inoculation	Moisture	LL (mm)	LL/Ht	LD (mm)	OL (mm)	OL/Ht	OD (mm)
GH	N	21.73 (2.48) d	0.50 (0.12) b	1.51 (0.57) b	27.27 (4.98) c	0.63 (0.19) c	0.46 (0.75) b
	MD	24.06 (6.59) d	0.56 (0.19) b	2.31 (0.55) b	28.71 (12.31) c	0.67 (0.22) bc	0.61 (0.79) b
	SD	22.47 (6.50) d	0.73 (0.78) b	0.40 (0.51) b	27.64 (12.16) c	0.63 (0.94) bc	0.59 (0.93) b
LT	N	27.97 (5.65) c	0.65 (0.21) b	2.44 (0.56) a	39.37 (9.22) b	0.93 (0.37) ab	0.82 (1.85) a
	MD	24.06 (10.64) a	0.56 (0.44) a	1.58 (0.60) a	54.52 (7.32) a	1.32 (0.47) a	0.67 (1.85) a
	SD	38.27 (11.60) b	1.04 (0.46) a	2.14 (0.47) a	44.39 (12.65) b	1.20 (0.48) a	2.57 (0.54) d
WM	N	12.73 (1.52) e	0.28 (0.09) c	0.64 (0.34) c	12.75 (3.43) d	0.28 (0.13) d	0.40 (0.01) c
	MD	12.67 (3.57) e	0.26 (0.09) c	0.72 (0.33) cd	12.34 (4.83) d	0.25 (0.12) d	0.54 (0.01) c
	SD	12.73 (3.30) e	0.43 (0.67) c	0.78 (0.46) cd	13.04 (4.23) d	0.44 (0.70) d	0.56 (0.01) c
W	N	12.73 (3.54) e	0.28 (0.10) c	0.64 (0.31) cd	12.75 (4.31) d	0.28 (0.12) d	0.54 (0.01) c
	MD	12.52 (3.73) e	0.27 (0.10) c	0.62 (0.35) d	11.82 (4.70) d	0.25 (0.11) d	0.43 (0.01) c
	SD	12.42 (3.52) e	0.46 (0.70) c	0.60 (0.26) d	11.12 (5.00) d	0.42 (0.69) d	0.42 (0.01) c

GH: *Grosmannia huntii*, LT: *Leptographium terebrantis*, WM: Wound + media, W: Wound, N: Normal watering, MD: Medium drought, SD: Severe drought, LL: Lesion length, LL/Ht: Lesion length Seedling height^{-1}, LD: Lesion depth, OL: Occlusion length, OL/Ht: Occlusion length Seedling height^{-1}, OD: Occlusion depth. Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$

Table 1.4 Effects of family and inoculation on the lesion and occlusion length.

		Lesion length (mm)		Occlusion length (mm)	
Family	Inoculation	*n*		*n*	
Tolerant	*G. huntii*	58	21.45 (3.65) c	58	25.78 (5.94) b
	L. terebrantis	50	35.22(10.24) b	50	43.62 (12.91) a
	Wound	58	12.74 (3.51) d	58	11.93(4.79) c
	Wound + media	55	12.76 (2.99) d	55	12.65 (4.17) c
Susceptible	*G. huntii*	58	24.03 (6.84) c	58	29.95 (10.61) b
	L. terebrantis	55	40.41 (12.99) a	55	49.45 (12.87) a
	Wound	54	12.34 (3.65) d	54	11.78 (4.64) c
	Wound + media	51	13.0 (2.92)d	51	4.16 (4.21)c

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.5 Effect on family x watering treatment x inoculation on lesion depth.

Family	Watering treatment	Inoculation	*n*	Lesion depth (mm)
Tolerant	Normal	*G. huntii*	18	1.41 (0.70) bcd
		L. terebrantis	14	2.55 (0.59) a
		Wound	20	0.62 (0.38) ef
		Wound + media	19	0.89 (0.38) cde
	Moderate drought	*G. huntii*	19	1.65 (0.44) ab
		L. terebrantis	19	2.29 (0.70) a
		Wound	19	0.50 (0.23) f
		Wound + media	16	0.80 (0.29) ef
	Severe drought	*G. huntii*	21	1.34 (0.51) bcd
		L. terebrantis	17	2.16 (0.49) a
		Wound	19	0.66 (0.27) ef
		Wound + media	20	0.69 (0.36) ef
Susceptible	Normal	*G. huntii*	19	1.60 (0.42) ab
		L. terebrantis	15	2.35 (0.54) a
		Wound	15	0.66 (0.19) ef
		Wound + media	14	0.85 (0.30) def
	Moderate drought	*G. huntii*	18	1.51 (0.65) bc
		L. terebrantis	19	2.33 (0.50) a
		Wound	18	0.75 (0.42) ef
		Wound + media	16	0.65 (0.35) ef
	Severe drought	*G. huntii*	21	1.45 (0.51) bc
		L. terebrantis	21	2.13 (0.46) a
		Wound	21	0.55 (0.24) ef
		Wound + media	21	0.87 (0.53) def

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.6 Effect of family and inoculation on seedling volume change (SVC).

Family	Inoculation	*n*	SVC (mm^3)
Tolerant family	*G. huntii*	62	11.86 (8.51) ab
	L. terebrantis	56	10.93 (6.28) abc
	No wound	57	16.97 (10.59) a
	Wound	61	16.88 (10.76) a
	Wound + media	63	14.47 (8.66) a
Susceptible family	*G. huntii*	61	7.67 (5.52) dc
	L. terebrantis	59	8.25 (5.84) bcd
	No wound	59	8.32 (7.45) d
	Wound	60	8.18 (6.13) dc
	Wound + media	59	8.50 (5.34) bcd

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.7 Effect of watering treatment and inoculation on increment in number of bud outbreak (BP).

Watering	Inoculation	*n*	BP
Normal water	*G. huntii*	40	2.23 (2.15) ab
	L. terebrantis	36	1.78 (1.51) abc
	No wound	41	2.90 (1.89) ab
	Wound	36	2.39 (2.05) ab
	Wound + media	39	2.28 (1.59) abc
Severe drought	*G. huntii*	42	1.17 (1.10) c
	L. terebrantis	36	1.50 (1.42) abc
	No wound	30	1.80 (2.41) abc
	Wound	41	1.95 (2.51) abc
	Wound + media	44	1.98 (1.64) abc
Medium drought	*G. huntii*	41	1.61 (1.84) abc
	L. terebrantis	41	1.56 (1.14) bc
	No wound	41	2.85 (2.57) a
	Wound	43	2.07 (2.16) abc
	Wound + media	41	1.85 (1.35) abc

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.8 Inoculation x family interaction on seedling height, stem dry matter and needle dry matter yield.

		Height growth (Ht)		Stem dry matter yield (Sy)		Needle dry matter yield (Ny)	
Inoculation	Family	*n*	cm	*n*	(g)	*n*	(g)
G. huntii	T	63	20.7 (10.5) abc	43	2.89 (1.68) c	51	3.47 (2.52) abc
	S	63	16.0 (10.6) d	43	2.15 (1.27) c	41	3.13 (2.10) abc
L. terebrantis	T	56	20.3 (8.1) abcd	46	2.98 (1.73) bc	48	3.16 (1.76) bc
	S	60	15.9 (10.7) d	46	2.52 (1.39) c	55	2.72 (2.41) c
Wound	T	63	25.2 (10.6) a	41	3.96 (1.24) ab	40	4.46 (2.98) ab
	S	60	18.3 (8.9) cd	40	2.60 (1.41) c	39	3.23 (2.44) abc
Wound + media	T	64	23.8 (9.8) abc	43	0.46 (2.03) abc	43	4.60 (2.30) ab
	S	62	17.9 (12.6) bcd	42	2.30 (1.09) c	44	3.15 (1.71) abc
No wound	T	58	26.6 (10.1) ab	42	4.35 (2.35) a	44	4.99 (3.99) a
	S	60	18.2 (10.6) d	44	2.30 (1.09) c	45	3.07 (2.79) bc

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.9 Inoculation x family interaction on fine and coarse root dry matter yield.

		Fine root dry matter yield (Fry)		Coarse root dry matter yield (Cry)	
Inoculation	Family	*n*	(g)	*n*	(g)
G. huntii	Tolerant	43	0.58 (0.61) ab	43	0.88 (0.65) abcd
	Susceptible	41	0.33 (0.41) b	41	0.51 (0.42) d
L. terebrantis	Tolerant	44	0.49 (0.44) b	44	1.20 (1.03) a
	Susceptible	47	0.35 (0.45) b	47	0.53 (0.38) d
Wound	Tolerant	41	0.87 (0.95) a	41	1.06 (0.67) ab
	Susceptible	41	0.52 (0.52) ab	41	0.57 (0.50) cd
Wound + media	Tolerant	43	0.60 (0.47) ab	43	0.98 (0.69) abcd
	Susceptible	41	0.40 (0.26) ab	41	0.61 (1.11) bcd
No wound	Tolerant	45	0.88 (0.92) a	45	1.04 (0.72) abc
	Susceptible	44	0.54 (0.68) ab	45	0.59 (0.58) cd

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.10 The effect of watering treatment x family interaction on seedling growth and biomass yields.

Drought	Family	*n*	Hty (cm)	*n*	Sy (g)	*n*	Ny (g)	*n*	Fry (g)	*n*	Cry (g)
N	T	101	25.6 (9.8) a	68	4.33 (2.21) a	77	4.36 (3.39) a	69	1.02 (0.87) a	69	1.27 (0.86) a
	S	97	18.3 (10.0) bc	67	2.87 (1.38) bc	71	3.45 (2.85) ab	66	0.53 (0.43) b	66	0.80 (0.99) bc
MD	T	106	25.4 (8.9) a	76	3.53 (1.84) ab	79	4.43 (2.94) a	75	0.59 (0.70) ab	75	1.13 (0.80) ab
	S	106	18.5 (10.4) bc	75	2.62 (1.41) c	78	3.33 (2.21) ab	77	0.51 (0.61) b	77	0.56 (0.39) cd
SD	T	97	18.6 (10.1) b	72	2.74 (1.29) bc	70	3.40 (1.85) ab	72	0.46 (0.43) bc	72	0.70 (0.49) c
	S	102	15.0 (11.5) c	73	1.86 (1.18) d	75	2.35 (1.63) b	71	0.25 (0.32) c	72	0.34 (0.29) d

N: Normal watering, MD: Medium watering, SD: Severe drought, Hty: Height growth, Sy: Stem dry yield increase, Ny: Needle dry yield increase, Fry: Fine root dry yield increase, Cry: Coarse root dry yield increase. Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.11 The effect of watering treatment x inoculation interaction on fine root dry matter yield (Fry).

Watering	Inoculation	*n*	Fine root dry yield (g)
Normal water	*G. huntii*	26	0.80 (0.56) a
	L. terebrantis	27	0.60 (0.49) ab
	No wound	32	0.78 (0.81) ab
	Wound	24	1.17 (1.09) a
	Wound + media	26	0.60 (0.39) ab
Severe drought	*G. huntii*	30	0.24 (0.32) bc
	L. terebrantis	32	0.17 (0.33) c
	No wound	23	0.52 (0.43) ab
	Wound	30	0.49 (0.47) ab
	Wound + media	28	0.40 (0.30) ab
Medium drought	*G. huntii*	28	0.38 (0.55) ab
	L. terebrantis	32	0.52 (0.42) ab
	No wound	34	0.78 (1.02) a
	Wound	28	0.51 (0.55) ab
	Wound + media	30	0.51 (0.45) ab

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.12 The effect of family x watering treatment x inoculation interaction on coarse root dry matter yield (Cry).

Family	Watering	Inoculation	*n*	Coarse root yield (g)
Tolerant	Normal	*G. huntii*	14	1.23 (0.72) abc
		L. terebrantis	13	1.80 (1.32) a
		No wound	18	1.09 (0.66) abc
		Wound	11	1.33 (0.70) abc
		Wound + media	13	1.00 (0.63) abc
	Severe drought	*G. huntii*	15	0.68 (0.55) bc
		L. terebrantis	15	0.59 (0.25) bc
		No wound	12	0.55 (0.42) bc
		Wound	15	0.77 (0.40) bc
		Wound + media	15	0.87 (0.71) abc
	Moderate drought	*G. huntii*	14	0.73 (0.58) bc
		L. terebrantis	16	1.28 (0.95) abc
		No wound	15	1.37 (0.81) ab
		Wound	15	1.16 (0.78) abc
		Wound + media	15	1.07 (0.76) abc
Susceptible	Normal	*G. huntii*	12	0.84 (0.46) abc
		L. terebrantis	14	0.45 (0.35) bc
		No wound	14	0.74 (0.75) bc
		Wound	13	0.97 (0.64) abc
		Wound + media	13	1.05 (1.92) abc
	Severe drought	*G. huntii*	15	0.33 (0.28) c
		L. terebrantis	17	0.42 (0.38) c
		No wound	12	0.31 (0.29) c
		Wound	15	0.33 (0.26) c
		Wound + media	13	0.29 (0.19) c
	Moderate drought	*G. huntii*	14	0.42 (0.37) c
		L. terebrantis	16	0.71 (0.35) bc
		No wound	19	0.65 (0.55) bc
		Wound	13	0.44 (0.28) bc
		Wound + media	15	0.50 (0.20) bc

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.13 Shoot-to-root and coarse-to-fine-root dry matter yield ratio (Sy/Ry) of different families.

Family	*n*	Sy/Ry	*n*	Cry/Fry (log)
Tolerant	202	1.56 (0.62) a	202	0.49 (1.09) a
Susceptible	198	1.87 (0.73) b	184	0.26 (0.96) b

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.14 Coarse-to-fine-root dry matter yield ratio (Cry/Fry) of different inoculation treatment.

Inoculation	*n*	Cry/Fry (log)
G. huntii	70	0.33 (1.07) ab
L. terebrantis	83	0.68 (1.10) a
No wound	81	0.33 (1.02) ab
Wound	77	0.24 (0.98) b
Wound + media	75	0.27 (0.94) ab

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.15 Effect of different watering treatment on shoot-to-root dry matter yield ratio (S/R).

Watering treatment	*n*	S/R (log)
Normal watering	130	1.51 (0.69) b
Medium drought	141	1.72 (0.70) a
Severe drought	129	1.89 (0.63) a

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.16 Effect of watering treatment x inoculation on needle-to-fine-root dry matter yield ratio (Ny/Fry).

Watering treatment	Inoculation	*n*	Ny/Fry (log)
Normal watering	*G. huntii*	24	1.43 (1.21) c
	L. terebrantis	26	1.61 (0.96) c
	No wound	29	1.83 (1.35) bc
	Wound	23	1.69 (0.92) bc
	Wound + media	25	2.00 (1.07) abc
Severe drought	*G. huntii*	22	2.52 (1.38) ab
	L. terebrantis	25	2.69 (1.01) a
	No wound	20	1.96 (0.58) abc
	Wound	26	1.84 (0.88) abc
	Wound + media	24	1.98 (0.76) abc
Medium drought	*G. huntii*	21	2.16 (0.56) abc
	L. terebrantis	31	1.92 (0.83) abc
	No wound	32	1.87 (1.14) abc
	Wound	21	2.34 (0.87) abc
	Wound + media	27	2.10 (0.77) abc

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.17 The effect of inoculation treatment on needle greenness.

Inoculation treatment	*n*	Needle greenness
G. huntii	64	42.21 (10.62) b
L. terebrantis	63	41.48 (1.96) b
No wound	62	50.76 (8.27) a
Wound	61	51.71 (10.04) a
Wound + media	65	52.19 (10.10) a

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.18 The effect of inoculation treatment interaction on water potential (Ψpd).

Inoculation treatment	*n*	Ψpd (-Mpa)
G. huntii	19	7.53 (2.37) a
L. terebrantis	18	7.52 (1.95) a
No wound	21	5.12 (1.49) b
Wound	15	5.92 (2.32) ab
Wound + media	19	6.29 (1.53) ab

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.

Table 1.19 The effect of watering treatments on relative water content.

Watering treatment	*n*	Relative water content (RWC)
Normal water	47	84.18 (10.63) a
Severe drought	51	82.48 (8.19) a
Moderate drought	52	76.79 (9.86) b

Means followed by the standard deviation in parenthesis. Numbers followed by different letters within a column indicate significant differences at $\alpha = 0.05$.