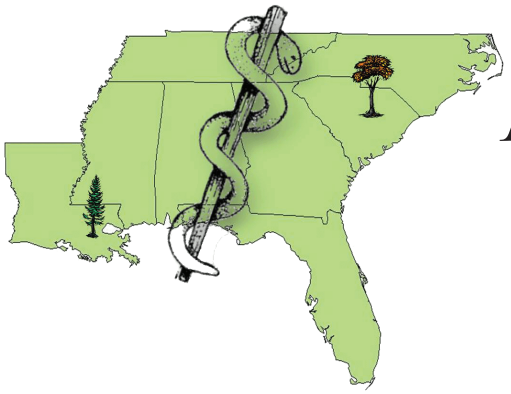

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INTERACTION OF SIMULATED RAINFALL TREATMENTS ON LOBLOLLY PINE SEEDLINGS INOCULATED WITH ROOT INFECTING OPHIOSTOMATOID FUNGI

by
Jeff Chieppa, Art Chappelka, and Lori Eckhardt

ABSTRACT
Seedlings from four families of loblolly pine (*Pinus taeda* L.) grown in capped open-top chambers were exposed to three different weekly moisture regimes. Moisture regimes varied in intensity and frequency of irrigation events, and were the same in the amount of irrigation received. Two of the families were selected for their tolerance to root infecting ophiostomatoid fungi, while the other two were selected for their susceptibility. Seedlings were inoculated with five inoculation treatments: no wound, wound only, wound+media, *Grosmannia huntii* and *Leptographium terebrantis*. After 13 weeks of moisture and inoculation treatments, seedlings were harvested. Seedling volume dry matter, yield, relative needle greenness, water potential and lesion characteristics were measured and analyzed for treatment effects using ANOVA procedures. Moisture stress increased susceptibility to inoculation in one loblolly pine family, however the increase was not specific to either root infecting ophiostomatoid fungi. In general, seedlings irrigated 7 days.week^{-1} showed greater chlorophyll content and growth compared to seedlings irrigated 4 days.week^{-1}. Seedlings irrigated 3 days.week^{-1} appeared to begin to reduce metabolic functions as confidence intervals were larger than other moisture treatments. In conclusion, moisture had no effect on the virulence of the fungi.

INTRODUCTION
Southern Pine Decline (SPD) is the term attributed to the premature death of *Pinus* spp. in the Southern U.S. due to a series of biotic and abiotic factors (Harrington and Cobb 1983, Otrosina et al. 1997, Eckhardt et al. 2007). These factors include associated root pathogenic fungi (*Leptographium* and *Grosmannia* spp.) and their root-feeding beetle vectors (*Hylastes salebrosus*, *H. tenuis*, *Hylobius pales*, and *Pachylobius picivorus*). Predisposing abiotic factors include resource stress (nutrient deficiencies, edaphic factors, and moisture stress), management strategies such as overstocking, mechanical injury and prescribed burning (Eckhardt et al. 2010). Studies have shown that when loblolly pine is inoculated with *Leptographium terebrantis* and, the fungus can result in the development of lesions in the phloem and resin-soaking in the xylem (Wingfield 1983, Eckhardt et al.2004, Matusick and Eckhardt 2010). *Grosmannia huntii* is a related fungal pathogen and reported to be more virulent on young pine seedlings when compared to *L. terebrantis* (Matusik and Eckhardt 2010).

Future climate change scenarios may play a significant role in the predisposing factors associated

with SPD. A comparison of two global circulation climate models indicate an increase in mean temperature globally (MacCracken et al. 2000), although the rate of increase is uncertain (MacCracken et al. 2000, IPCC 2013). Another uncertainty is how much precipitation will occur in the Southeastern U.S. in the next 50-100 years (MacCracken et al. 2000, IPCC 2013). Observed precipitation patterns over the past 60 years may indicate future rainfall for a region. For example, in 2007 the worst drought in 100 years occurred in the Southern U.S. and was followed by flooding in 2009 (Wang et al. 2010). While changes in the intensity and frequency of summer precipitation may continue in the Southeastern U.S., there is still debate as to its underlying cause (Wang et al. 2010, Li et al. 2011, Seager et al. 2009). Another trend in precipitation patterns has been the daily variation in precipitation events where storms are occurring less frequently but are characterized by more intense rainfall for longer durations in North America (Kunkel et al. 2013, Muschinski and Katz 2013, IPCC 2013).

A concern when considering future precipitation patterns is how forests will respond to altered drying and wetting periods (Hanson and Weltzin 2000, MacCracken et al. 2000). Trees may thrive during wetter periods and experience moisture stress if evaporative losses increase during warmer, drier periods (Neilson and Drapek 1998). Changes in precipitation patterns can have cascading effects throughout forest ecosystems. Droughts can reduce tree vigor and alter insect and pathogen physiology (Dale et al. 2001). The body of literature on this subject is extensive (Desprez-Loustau et al. 2006), however the effects of precipitation changes are anticipated to be unique based on the host's and pathogen's physiology (Rouault et al. 2006, Sturrock et al. 2011).

The impact that changes in precipitation will have on insect population in the Southeastern U.S. needs to be understood, specifically for those insects that vector pathogenic fungi (e.g. ophiostomatoid fungi) (Kirisits 2004, Bentz et al. 2010). One example is the influence of drought stress and its impact on host trees and subsequent influence on bark beetle populations. It has been shown that an increase in drought stress of the host tree results in greater infestations of bark beetles (Jones et al. 2001, Jactel et al. 2012, Klepzig et al. 2004, Koricheva et al. 1998). Host stress is only a single factor in this interaction, as insect physiology and ecology shifts can have effects on the host-insect relationship as well (Clarke and Fraser 2004, Gillooly et al. 2001).

Numerous studies have examined the effects of precipitation changes on plants and fungal pathogen interactions. These studies usually compare a sufficiently watered control and a reduced water treatment (Seiler and Johnson 1988, Goheen et al. 1978, Croisé et al. 2001, Meier et al. 1990, Matusick et al. 2008). While these studies provide insight as to host plant responses to periods of reduced moisture availability, less is known as to the impact that fluctuating moisture availability will have on host-pathogen interactions. Some studies indicate that fluctuating moisture availability results in decreased productivity of loblolly pine (Tschaplinski et al. 1993).

In an assessment of the effect of potential future climate change scenarios for the Southeastern U.S., Jones et al. (2001) stated that changes in water availability are important and require further investigation. Water availability can cause alterations in loblolly pine vigor resulting in biotic organisms, such as *L. terebrantis* or *G. huntii*, potentially exacerbating declines and reducing productivity.

The overall objective of this study was to elucidate the interactions of two root infecting ophiostomatoid fungi (*L. terebrantis* and *G. huntii*) in the presence of climatic conditions predicted in the next 50 to 100 years in the Southeastern U.S. More specifically, our main aim was to understand how changes in precipitation patterns may affect loblolly pine infected with the root infecting ophiostomatoid fungi. The hypotheses tested include: (1) loblolly pine will become more susceptible to *L. terebrantis* and *G. huntii* as the irrigation regime is altered in intensity and frequency; and (2) loblolly pine families selected for their tolerance to root infecting ophiostomatoid fungi would be more tolerant to changes in the intensity and frequency.

MATERIALS AND METHODS

Study Site and Capped Open-Top Chamber

The research site (approximately 0.02 km^2 in area) is located approximately 5 km North of Auburn University Campus, Auburn, AL, U.S. The site contained 24 open-top chambers (OTCs), monitoring sheds and a small laboratory. The OTCs were 4.8 m height x 4.5 m diameter aluminum framed structures with fans (1.5 horse-power motors) and chamber plastics (Gilliland et al. 2012). Plastic caps were attached to each OTC to exclude ambient rainfall and permitted adequate airflow (Heagle et al. 1989).

Prior to the commencement of the study (March 2014), the vegetation growing in each OTC was killed with a 3% solution of glyphosate. Once dead, the vegetation was removed prior to the ground being covered with landscape fabric to prevent further unwanted vegetation growth within each OTC.

Seedlings

Bareroot seedlings from four commercially grown loblolly pine families were used for this study (lifted/extracted from the nursery in November 2013). Based on previous studies, two of these loblolly pine families were considered “tolerant” (T1 and T2) and two “susceptible” (S1 and S2) to ophiostomatoid root infecting ophiostomatoid fungi (Singh et al. 2014). In January 2014, 2700 seedlings (750 per family) were planted in trade gallon pots with ProMix BX® peat-based potting mix (Premier Tech, Quebec, Canada). Seedlings were kept under a shade house and watered daily for 17 weeks until being deployed into the OTCs in May 2014.

Irrigation Treatments

To determine the longest duration the potting mix could go before difficulty rewetting, eight seedlings (two from each family) were placed in a greenhouse at approximately 90 °F (~32 °C). After 3 days of water being withheld, the potting mix became dried out and therefore, the longest period between irrigation events was set at 2 days.

Three simulated precipitation treatments were used (with each treatment having 3 replicates). The treatments were as follows: (1) irrigated 3 $days.week^{-1}$ (3D) during the experimental period, (2) irrigated 4 $days.week^{-1}$ (4D) during the experimental period, (3) irrigated daily (7D). Irrigation nozzles within each OTC were adjusted to ensure an even water distribution and flow rates within and between the chambers. These were adjusted to ensure 58 minutes of irrigation resulted in 1 inch or 25.4 mm of precipitation. While the days of irrigation varied between treatments, each chamber received the same amount of precipitation at the end of each week (Table 3.1). Weekly

irrigation values were estimated based on the 30-yr (1971-2000) average precipitation for Auburn, AL. In June 2014 a 20% increase was given to all treatment amounts to compensate for higher temperatures and increased airflow in the chamber (Table 3.2). Monitoring throughout June indicated this adjustment approximately offset the increased loss of moisture in the chambers.

Inoculations

Stem inoculations were conducted as described by Nevill et al. (1995) in May 2014 using the wound+inoculum method. Five inoculation treatments were used in this study: no wound (NW), wound only (W), wound+media (WM), *L. terebrantis* (LT) and *G. huntii* (GH). To inoculate seedlings, a sterile razor blade was used to cut a 5 cm vertical lesion into the bark 5 cm above the soil line. Plugs of 2% malt extract agar (MEA, 3 mm) were placed into the wound. Media was either sterile or had cultures of *L. terebrantis* (LT) or *G. huntii* (GH) growing on it. Seedling stem wounds were wrapped in cotton dampened with deionized water and then wrapped in Parafilm® to prevent desiccation of the MEA and avoid contact with other biological contaminants.

Measurements and Harvest

Root collar diameter (RCD) and height measurements were recorded for all seedlings at both the study initiation (February 2014) and completion (August 2014). Seedling volume change was calculated ($Volume_{Final} - Volume_{Initial} = Volume_{Change}$) to determine overall growth for individual seedlings. The equation $Volume = RCD^2 \; x \; height$ was used to estimate seedling volume (Ruehle et al. 1984).

During planting in February, 40 seedlings from each family were destructively harvested into needles (NE), shoot (SH), coarse roots (CR) and fine roots (FR). These components were placed in drying-ovens for 70 °C at 72 hours. At the conclusion of the study (August 2014), two seedlings from each treatment combination and chamber were selected for final dry matter seedling biomass. Initial family averages for each component (needles, shoots etc.) were subtracted to estimate dry matter yield.

Eleven seedlings from each treatment combination, from all nine OTCs, were examined for relative leaf chlorophyll using a SPAD-502 chlorophyll meter (Spectrum Tech. Inc., Plainfield, IL) during final harvest (August 2014). Needles from the first 2013 flush were selected as they had reached physiological maturity (Sasek et al. 1991).

The remaining two seedlings from each combination treatment, from each chamber, were sampled for water potential measurements using a Scholander pressure bomb (PMS Instrument Company, Albany, OR). Five cm of a lateral branch was cut off each seedling and sampled as described by Kaufmann (1968). Midday sampling occurred between 1300-1500 h. Predawn water potential sampling occurred between 0300- 0500 h. Seedlings were sampled on days of irrigation and days where irrigation was withheld, however, because of significant variation between replicates the data for predawn water potential on non-irrigated days were not analyzed and thus are not included in the analysis.

Another sample of 11 seedlings per treatment was measured for lesion characteristics. These seedlings were cut at the soil line and placed in plastic bins filled with FastGreen stain (FastGreen FCF; Sigma Chemical Co., U.S.) as described by Singh et al. (2014). After 72 hours, stems were

removed and the lesion length, width and depth were measured. Two pieces of stem tissue from each lesion were removed from the stem and plated on malt extract agar with cyclohexamide and streptomycin sulfate for fungal re-isolation (Singh et al. 2014).

Data Analysis

The experimental design was a split-split-split plot with replicates at all levels. Thee irrigation treatments, 4 loblolly pine families and 5 inoculation treatments produced 60 treatment combinations. Each treatment combination was replicated 15 times in each chamber at the beginning of the study. All statistical analyses were conducted using SAS (Version 9.3 SAS Institute, Inc. Cary, NC) and STATISTICA (Statsoft, Inc. Tulsa, OK). ANOVA procedures (Glimmex procedures), followed by post hoc Tukey (Honest Significant Difference – HSD) procedures were undertaken to further investigate treatment effects. Alpha was set at 0.05.

RESULTS

Needle Greenness

Seedlings in 3D and 4D chambers had no difference in needle greenness ($P = 0.418$). Seedlings in 7D treatments had 6.1% greener needles ($P < 0.0001$) compared to seedlings in 4D treatments, although 7D treatments were not different than 3D seedlings (Figure 3.1). Neither family nor inoculation treatments affected needling greenness ($P = 0.323$ and $P = 0.675$, respectively). Family S1 in 4D treatments had less needle greenness when compared to the same family in 7D treatments ($P = 0.033$), however, 3D seedlings were not different from 4D ($P = 0.883$) or 7D ($P = 0.991$) seedlings. There were no other significant interactions between treatments or treatment combinations (Table 3.3).

Seedling Volume Change

Seedlings grown in 7D treatments had a greater change in volume compared to 3D (0.7%, $P < 0.0001$) and 4D (1.2%, $P = 0.015$) treatments. Seedling volume changes in 3D and 4D treatments were not significantly different ($P = 0.060$) from each other. The T1 seedlings had the largest volume change ($P < 0.0001$) compared to S2 seedlings that had the least (11.7% less than T1, $P < 0.0001$). T2 was found to have the second largest change in seedling volume (1.5% less than T1, $P < 0.0001$), followed by S1 (8.6% less than T1, $P < 0.0001$). Inoculation had no effect on seedling volume changes ($P = 0.805$). S1 seedlings grew more in the 7D treatments compared to either the 3D (5.3%, $P < 0.0001$) or 4D (4.0%, $P < 0.001$) treatments. S2 was not significantly affected by irrigation treatment ($P > 0.999$). T1 seedlings grown in 3D treatments were found to have a greater volume change compared to seedlings in 4D (1.8%, $P < 0.001$) and 7D treatments (1.1%, $P < 0.001$). T1 seedlings in 4D and 7D changes were found to be not significantly different ($P = 0.999$, Figure 3.2). T2 seedlings grown in 4D treatments were not significantly different from those grown in 3D ($P = 0.510$) or 7D ($P = 0.262$) treatments, although T2 seedlings grown in 3D treatments were found to have greater volume changes compared to seedlings grown in 7D treatments (4.1%, $P = 0.023$). There were no other significant interactions between treatments or treatment combinations (Table 3.3).

Dry Matter Yield

Needle dry matter yield (DMY) and shoot DMY were found to be similar so those response variables were combined for analyses and named aboveground DMY (needles + shoots). Coarse

root DMY and fine root DMY were found to be similar so those were also combined for analysis and reported as belowground DMY (coarse roots + fine roots).

Aboveground DMY

Seedlings grown in 7D treatments had 7.1% greater ($P = 0.010$) aboveground DMY compared to seedlings grown in 4D treatments (7.1%), however, there was no difference ($P = 0.188$) between 4D and 3D aboveground DMY. The two tolerant families of loblolly pine had no difference in DMY ($P = 0.051$) when compared. Likewise, S1 and S2 seedlings were not significantly different in aboveground DMY ($P = 0.230$), however, both had significantly less ($P < 0.0001$) aboveground DMY compared to T1 and T2 seedlings (22.4% and 27.5% less than T1). Inoculation treatment had no significant effect on aboveground DMY ($P = 0.192$). Families S2, T1 and T2 DMY was not significantly affected by irrigation treatment ($P = 1.000$, $P > 0.659$, $P > 0.728$ respectively). S1 seedlings grown in 7D treatments had greater aboveground DMY compared to both 3D (20.8%) and 4D (22.9%) treatments ($P < 0.002$). There were no other significant interactions between treatments or treatment combinations (Table 3.3).

Belowground DMY

Seedlings grown in 3D treatments were found to have no significant difference in belowground DMY compared to seedlings grown in 4D ($P = 0.569$) and 7D ($P = 0.085$) treatments, however, seedlings grown in 7D treatments had 8.4% greater belowground DMY compared to seedlings grown in 4D treatments ($P = 0.005$). T1 and T2 seedlings had the greatest belowground DMY, although were not significantly different from each other ($P = 0.442$). S1 and S2 seedlings belowground DMY were not significantly different ($P = 0.229$), and both S1 and S2 seedlings had less belowground DMY compared to both T1 and T2 (32.9% and 38.5% compared to T1, $P < 0.0001$). Inoculation had no significant effect on belowground DMY ($P = 0.064$). There were no other significant interactions between treatments or treatment combinations (Table 3.3).

Total DMY

Seedlings grown in 3D treatments were found to have no difference in total DMY compared to seedlings grown in 4D ($P = 0.636$) and 7D ($P = 0.073$) treatments, however, seedlings grown in 7D treatment were found to have greater total DMY compared to seedlings grown in 4D treatments (7.4%, $P = 0.005$, Figure 3.3). T1 and T2 seedlings were found to have the greatest total belowground DMY, although were not different from each other ($P = 0.072$). Seedling families S1 and S2 total DMY were not different from each other ($P = 0.211$), and both S1 and S2 seedlings were found to have significantly less belowground DMY when compared to both “tolerant” families (24.3% and 29.5% less than T1 respectively, $P < 0.0001$). Inoculation had no effect on total DMY ($P > 0.120$). Families S2, T1 and T2 were not significantly affected for total DMY by treatments. S1 seedlings grown in 7D treatments had significantly greater total DMY compared to seedlings grown in 3D (20.4%) and 4D (22.3%) treatments ($P < 0.003$), however, 3D and 4D seedlings were not different from each other ($P = 1.000$). There were no other significant interactions between treatments or treatment combinations (Table 3.3).

Predawn Water Potential on Irrigated Days

Seedlings grown in 4D treatments had lower water potentials compared to seedling grown in 3D (15.7%) and 7D (20.8%) treatments ($P < 0.001$), however, there was no difference in predawn water potential between seedlings grown in 3D and 7D treatments ($P = 0.395$). Family and

inoculation each had no effect on water potential ($P > 0.907$). Predawn water potential was the same for families S1, T1 and T2 and were not affected by irrigation treatment ($P > 0.143$). S2 seedlings grown in 3D treatments were not significantly affected compared to seedlings grown in 4D ($P = 0.956$) or 7D ($P = 0.089$) treatments, however, seedlings grown in 4D treatments were more water stressed compared to those grown in 7D treatments (35.9%, $P < 0.001$). There were no other significant interactions between treatments or treatment combinations (Table 3.3).

Midday Water Potential on Irrigated Days

Seedlings grown in 3D treatments were more water stressed compared to seedlings grown in 4D (10.7%) and 7D (9.4%) treatments ($P < 0.001$, Figure 3.4), however, there was no difference between the 4D and 7D treatments ($P = 0.702$). There were no significant difference ($P = 0.917$) in midday water potential values between seedling families. Seedlings inoculated with GH were more water stressed than the NW (9.2%) and WM (9.6%) controls ($P < 0.040$). The water potentials of the four seedling families were not affected by inoculation treatment ($P = 0.153$) or irrigation treatment ($P = 0.574$). Wound only seedlings, LT and GH had no difference in water potential between irrigation treatments ($P > 0.221$). Seedlings that were not inoculated (NW) grown in 7D treatments were not different from NW seedlings grown in 3D and 4D treatments ($P > 0.599$). Wound+media seedlings grown in 7D treatments were not different from seedlings grown in 3D and 4D treatments ($P > 0.149$), however, WM seedlings grown in 3D treatments had lower water potentials (18.6%) compared to those grown in 4D treatments ($P = 0.048$). There were no other significant interactions between treatments or treatment combinations (Table 3.3).

Midday Water Potential on Non-Irrigated Days

Seedlings grown in 7D treatments were less water stressed than those grown in 3D or 4D treatments (19.1% and 12.8% respectively, $P < 0.003$), however, seedlings grown in 3D treatments and 4D treatments were not significantly different from each other ($P = 0.143$, Figure 3.4). There was no difference in water potentials between seedling families ($P = 0.361$) or inoculation treatments ($P = 0.412$). Families S1, T1 and T2 water potentials values were not affected by irrigation treatment ($P > 0.070$). S2 seedlings grown in 4D treatments were not different than seedlings grown in 3D and 7D treatments ($P > 0.424$), however, S2 seedlings grown in 3D treatments were more water stressed than those grown in 7D treatments (25.1%, $P = 0.009$). There were no other significant interactions to report between treatment or treatment combinations (Table 3.3).

Lesion Length and Lesion Length/Seedling Height

Lesion length was unaffected by irrigation treatment ($P = 0.905$). S1, T1 and T2 overall had no difference in lesion length ($P > 0.563$). Seedlings inoculated with control treatments (W and WM) had similar lesion lengths ($P = 0.827$). Similarly seedlings inoculated with LT and GH had similar lesion lengths ($P = 0.729$). Both GH and LT inoculated seedlings had significantly larger lesions compared to the controls (8.1% and 8.3% respectively, $P < 0.0001$).

All families inoculated with W and WM controls were found to have no differences in lesion length ($P > 0.983$). Putatively susceptible seedlings in family S1 that were inoculated with LT and GH had larger lesion lengths compared to the non-inoculated seedlings within that family (8.1% and 10.0% respectively, $P < 0.0001$). The family S2 seedlings inoculated with LT and GH had larger lesion lengths compared to the controls (12.9% and 9.6% respectively, $P < 0.0001$), however,

lesions produced by the fungi were not significantly different from each other (P = 0.097). T1 seedlings with LT and GH had larger lesions when compared to the non-inoculated control (5.2% and 6.0% respectively, $P < 0.0001$). There was no difference in the fungi used in the trial as both LT and GH lesion lengths were similar on the T1 family (P = 1.000). Likewise the putatively tolerant family seedlings inoculated with the LT and GH treatments had larger lesions when compared to the non-inoculated controls (6.7% for both, $P < 0.0001$). There was no difference in virulence among the two fungi as both LT and GH lesion lengths were similar on family T2 (P = 1.000).

Lesion length ratio (lesion length.seedling height^{-1}) was greatest in the S2 family and lowest in the T1 family (9.2% less than S2, $P < 0.0001$). S1 lesion length ratio was smaller than the lesion length ratio on the S2 family seedlings (2.6%, $P < 0.001$). T2 lesion length ratio was lower than S2 seedlings (7.8%, $P < 0.0001$). Irrigation treatment had no effect on seedling lesion length ratios (P = 0.307). Lesion length ratio for W and WM seedlings were similar for both wounds (P = 0.991). Likewise, the two fungal inoculations (GH and LT) were similar to each other (P = 0.808). Seedlings inoculated with GH and LT had lesion length ratios 7.0% and 7.5% greater than the non-inoculated control average ($P < 0.0001$). S2, T1 and T2 seedling lesion length ratios were not affected by the various irrigation treatments ($P > 0.406$). S1 seedlings in 7D treatments had smaller lesion length ratios compared to S1 seedlings in 3D treatments (5.1%, P = 0.026) (Table 3.3).

Lesion Volume and Lesion Volume/Seedling Volume

When examining the effect of the fungal inoculations on the four seedling families lesion volume was greatest in the tolerant T1 and T2 families (P = 0.891). For families S1 and S2 lesion volumes were similar to each other (P = 0.637) and smaller than the lesion volumes on the T1 and T2 seedling families (3.7% and 2.5% less than T1 respectively, $P < 0.005$).

The various irrigation treatments had no effect on lesion volume (P = 0.110). Wound only seedlings had the smallest lesion volumes in contrast to LT inoculated seedlings which had the largest lesion volumes (15.8% larger than W, $P < 0.0001$). Wound+media (WM) and GH seedlings were found to have the largest lesion volumes (4.9% and 14.4% respectively) compared to W seedlings ($P < 0.0001$).

Lesion volume ratio was not significantly different among the two families T1 and T2 (P = 0.384). S1 and S2 ratios were less than those of T1 and T2 (16.4% and 21.1% less than tolerant families' average, $P < 0.0001$). Irrigation treatments had no effect on lesion length ratio (P = 0.145). Seedlings inoculated with LT had the largest lesion volume ratios (28.2%, 23.2% and 4.1% greater than W, WM and GH seedlings respectively, $P < 0.040$). Lesion volume ratios in GH seedlings were found to be greater than W and WM controls (25.1% and 19.8% greater than W and WM seedlings respectively, $P < 0.0001$), which were also different (WM were 6.6% greater than W seedlings, $P < 0.0001$). Family S2, T1 and T2 lesion volume ratios were not affected by the 3 different irrigation treatments ($P > 0.447$). S1 seedlings in 3D and 4D chambers were not different (P = 0.865), however, S1 seedlings in 3D chambers had greater lesion volume ratios than those grown in 7D chambers (12.8%, P = 0.004) (Table 3.3).

DISCUSSION

The alteration of irrigation patterns in the OTC to simulate precipitation changes due to climate change did not result in an increase in susceptibility of four commonly grown loblolly pine families to the root infecting ophiostomatoid fungi. One family did have an increase in injury from the wounding process but it was restricted to S1 and was not specific to either *L. terebrantis* or *G. huntii*. This indicates that some loblolly pine families do not necessarily become more susceptible to root infecting ophiostomatoid fungi, but may become more susceptible as a result of mechanical stress. Based on the results of Singh et al. (2014), it is difficult to say what host-pathogen interactions are creating the tolerance-susceptible spectrum in loblolly pine seedlings.

Water stress has been found to result in a decrease in net photosynthesis in loblolly pine (Samuelson et al. 2014) which is accompanied by a decrease in transpiration rate (Groninger et al. 1996). Seiler and Johnson (1988) found evidence that water stress conditioning allowed loblolly pine seedlings to photosynthesize at lower water potentials than usual that may explain why the 3D irrigation treatment was not different than the 4D and 7D treatments. In our study, 3D seedling responses had large 95% confidence intervals, which may indicate some seedlings had begun to reduce metabolic functions. This would likely causes some seedlings to decrease photosynthetic rates while others continue to actively photosynthesize, which in our study, caused an increase in uncertainty.

Loblolly pine has been shown to have reduced growth when exposed to moisture stress (Meier et al. 1990, Seiler and Johnson 1988, Tschaplinkski et al. 1993). The degree to which loblolly pine responds to moisture stress has been shown to be affected by the seed source location (Seiler and Johnson 1988). In these trials, only S1 had reduced growth given the water stress treatments for both dry matter yield and volume growth. In both tolerant families, dry matter yield was not affected, however, seedlings had greater volume growth when watered 3 days.week^{-1} compared to other treatments. Therefore, the response of loblolly pine to alterations in moisture availability will likely be family dependent. Given the results of this study, it is difficult to determine if there is a link between tolerances to root infecting ophiostomatoid fungi and drought.

In a previous study, it was observed that inoculation with root infecting ophiostomatoid fungi increased midday water stress when compared to non-inoculated control seedlings (Chapter 2). However, in this study, there was no moisture x inoculation interactions. Seedlings irrigated 3 days.week^{-1} were not able to recover after irrigation an event, which strengthens the idea that some seedlings had begun to reduce metabolic processes. Seedlings irrigated 4 days.week^{-1} were able to recover to water potentials similar to those in seedlings watered 4 days.week^{-1}.

CONCLUSIONS

The results of the study indicate that tolerance to root infecting ophiostomatoid fungi may be linked to moisture stress sensitivity. One of the two susceptible families used was also increasingly sensitive to moisture stress. The same family (S1) that was sensitive to changes in moisture also had a larger lesion or wound when irrigated less frequently. This was not specific to inoculation with the pathogenic fungi and therefore we reject the hypothesis that altered moisture availability increases loblolly pine susceptibility to root infecting ophiostomatoid fungi. We can conclude that the strategy to compensate for mechanical stress/wounding is compromised by moisture stress in some families of loblolly pine.

While most work concerning loblolly pine response to drought has not focused on seedlings (Graham et al. 2012, Murthy et al. 1996, Cregg et al. 1988), seedlings will be most likely to succumb to moisture stress (Allen et al. 2010) unless catastrophic drought occurs in the Southeastern U.S. While some studies have utilized seedlings (Seiler and Johnson 1988, Goheen et al. 1978, Croisé et al. 2001, Meier et al. 1990, Matusick et al. 2008), little is known how fluctuating water stress will affect host-pathogen interactions.

It is important to note that there are two overall precipitation patterns that are occurring in North America given the changing climate. The first is the year to decade variation in precipitation patterns that occur during the summer months in the Southeastern U.S. (Wang et al. 2010, Li et al. 2011, Seager et al. 2009, IPCC 2013). The second is the minute to daily variation in precipitation extremes (high moisture to low moisture) that is occurring (Westra et al. 2014). While the long term trends may contribute to pest and disease outbreaks and factor into forest decline, the short term variations in moisture availability will likely affect regeneration and understory species in forest ecosystems. It is important to consider the differences between precipitation patterns when designing experiments and considering the results. Distinguishing between the effects of altered moisture availability becomes increasingly important as multiple stresses, in our case root infecting ophiostomatoid fungi, are added to the design.

Based on several studies (Garrett et al. 2006, Sturrock et al. 2011, Manning and von Tiedemann 1995) there are three common relationships to look for when analyzing climate-host-pathogen relationships: (1) Climate can affect the pathogen's virulence, abundance, distribution and general biology/ecology; (2) climate can alter the host's defense, abundance, distribution and general biology/ecology; and (3) climate can change the way the host and pathogen interact, through direct or indirect effects. When applying this framework to insect-fungal disease relationships, examining multiple species-species interactions can become complex. It is important to understand the underlying physiological mechanisms for these interactions. For instance, this study focuses on host-fungal pathogen relationships to changes in irrigation (precipitiation) patterns that may occur with climate change. While bark beetles have been shown to capitalize on trees with reduced vigor caused by moisture stress (Jones et al. 2008, Jactel et al. 2012, Klepzig et al. 2004, Koricheva et al. 1998), there is little evidence to support increased pathogen virulence in moisture stressed trees (Goheen et al. 1978, Matusick et al. 2008, Joseph et al. 1998, Croisé et al. 2001). Further studies should examine the insect vector-climate interactions when investigating insect-fungal disease relationships.

LITERATURE CITED

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Table 3.1. Summary of irrigation treatments (minute quantities) by month

Month	Week	Days of Rain	Minutes/Day	Minutes/Week	Amount/Week (cm)
May	1	7D	9	63	2.8
		4D	15	60	2.6
		3D	21	63	2.8
	2	7D	9	63	2.8
		4D	15	60	2.6
		3D	21	63	2.8
June	3	7D	9	63	2.8
		4D	15	60	2.6
		3D	21	63	2.8
	4	7D	9	63	2.8
		4D	15	60	2.6
		3D	21	63	2.8
	5	7D	9	63	2.8
		4D	15	60	2.6
		3D	21	63	2.8
	6	7D	11	76	3.3
		4D	18	72	3.2
		3D	25	76	3.3
July	7	7D	14	101	4.4
		4D	25	101	4.4
		3D	32	97	4.3
	8	7D	14	101	4.4
		4D	25	101	4.4
		3D	32	97	4.3
	9	7D	14	101	4.4
		4D	25	101	4.4
		3D	32	97	4.3
	10	7D	14	101	4.4
		4D	25	101	4.4
		3D	32	97	4.3
	11	7D	14	101	4.4
		4D	25	101	4.4
		3D	32	97	4.3
August	12	7D	11	76	3.3
		4D	18	72	3.2
		3D	25	76	3.3
	13	7D	11	76	3.3
		4D	18	72	3.2
		3D	25	76	3.3

7D = irrigated 7 days.week^{-1}. 4D = irrigated 4 days.week^{-1}. 3D = irrigated 3 days.week^{-1}

Table 3.2. Summary of irrigation treatments (rainfall quantity) compared to the average precipitation for Auburn, AL (1971-2000) (AWIS, Inc.).

Month	Weeks	Days of Rain (chamber)	Total Irrigation (cm)	Monthly Average For Study (cm)	Monthly Average (1971-2000)	Monthly Average (1971-2000) + 20%
May	2	7D	5.5	11.0	9.7	N/A
		4D	5.2	10.4		
		3D	5.5	11.0		
June*	4	7D	10.9	10.9	10.3	10.9
		4D	11.0	11.0		
		3D	11.6	11.6		
July*	5	7D	22.1	17.7	14.9	17.9
		4D	22.1	17.7		
		3D	21.3	17.0		
August*	2	7D	6.6	13.2	9.2	11.0
		4D	6.3	12.6		
		3D	6.6	13.2		

"Weeks" indicates how many weeks irrigation was applied for the month. 7D = irrigated 7 days.week^{-1}. 4D = irrigated 4 days.week^{-1} (Sunday, Tuesday, Thursday, Saturday). 3D = irrigated 3 days.week^{-1} (Monday, Wednesday, Friday). The a*sterisks* denote months where a 20% increase was applied to all irrigation times (in minutes) in order to compensate for increased evaporation rates as a result of the fans and increased temperatures inside the capped open-top chambers. N/A denotes 'not applicable' as a 20% increase in irrigation time was not applied to the month of May.

Table 3.3a. ANOVA F-Test Values and *P*-Values by treatment (Irrigation, Family)

Measurement	n	ANOVA F-Test Values and *P*-Values by Treatment Combination					
		Irrigation			Family		
		df	F-value	*P*-Value	df	F-value	*P*-Value
Seedling Volume Change	2043	3	4.40	0.009*	3	197.76	< 0.0001***
Dry Matter_Total	294	3	4.74	0.08	3	96.04	< 0.0001***
Needles	298	3	2.54	0.009*	3	71.76	< 0.0001***
Shoots	298	3	4.79	0.017*	3	48.52	< 0.0001***
Aboveground (Ne+Sh)	298	3	4.11	0.012*	3	79.01	< 0.0001***
Coarse Roots	298	3	4.49	0.002*	3	124.83	< 0.0001***
Fine Roots	298	3	6.27	0.008*	3	43.12	< 0.0001***
Belowground (Cr+Fr)	298	3	4.96	< 0.0001***	3	122.14	< 0.0001***
SPAD	1561	3	11.63	0.90	3	1.16	0.323
Lesion Length	1217	3	0.10	0.17	3	5.12	0.002*
Lesion Volume	1215	3	1.76	0.31	3	10.98	< 0.0001***
Lesion Length/ Seedling Height	1194	3	1.81	0.14	3	71.88	< 0.0001***
Lesion Volume/ Seedling Volume	1192	3	1.93	< 0.0001***	3	90.83	< 0.0001***
Mid-Day Wet Water Potential	284	3	10.74	< 0.0001***	3	0.17	0.918
Mid-Day Dry Water Potential	281	3	14.09	< 0.0001***	3	1.07	0.361
Pre-Dawn Wet Water Potential	276	3	12.98	n/a	3	0.10	0.963
Pre-Dawn Dry Water Potential	n/a	n/a	n/a	0.00	n/a	n/a	n/a

* denotes $P < 0.05$; ** denotes $P < 0.001$; *** denotes $P < 0.0001$. F(df, n) denotes the degrees of freedom (df) and sample size (n) of the ANOVA F-Test. “n/a” denotes data were not analyzed.

Table 3.3b. ANOVA F-Test Values and *P*-Values by treatment (Inoculation, Family*Irrigation)

Measurement	n	ANOVA F-Test Values and P-Values by Treatment Combination					
		Inoculation			Family*Irrigation		
		df	F-value	*P*-Value	df	F-value	*P*-Value
Seedling Volume Change	2043	4	0.92	0.453	6	6.71	< 0.0001***
Dry Matter_Total	294	4	3.01	0.019*	6	3.86	< 0.001**
Needles	298	4	4.40	0.002*	6	4.61	< 0.001**
Shoots	298	4	0.46	0.767	6	2.26	0.037*
Aboveground (Ne+Sh)	298	4	2.77	0.027*	6	4.22	< 0.001**
Coarse Roots	298	4	1.46	0.215	6	2.58	0.019*
Fine Roots	298	4	4.28	0.002*	6	1.69	0.123
Belowground (Cr+Fr)	298	4	3.02	0.018*	6	1.58	0.151
SPAD	1561	4	0.58	0.675	6	0.5	0.832
Lesion Length	1217	3	84.40	< 0.0001***	6	0.84	0.538
Lesion Volume	1215	3	208.07	< 0.0001***	6	1.24	0.283
Lesion Length/ Seedling Height	1194	3	58.37	< 0.0001***	6	3.83	< 0.001**
Lesion Volume/ Seedling Volume	1192	3	137.93	< 0.0001***	6	3.8	< 0.001**
Mid-Day Wet Water Potential	284	4	3.19	0.014*	6	0.8	0.574
Mid-Day Dry Water Potential	281	4	0.99	0.412	6	1.58	0.152
Pre-Dawn Wet Water Potential	276	4	0.25	0.907	6	2.2	0.044*
Pre-Dawn Dry Water Potential	n/a	n/a	n/a	n/a	n/a	n/a	n/a

* denotes $P < 0.05$; ** denotes $P < 0.001$; *** denotes $P < 0.0001$. F(df, n) denotes the degrees of freedom (df) and sample size (n) of the ANOVA F-Test. “n/a” denotes data were not analyzed.

Table 3.3c. ANOVA F-Test Values and *P*-Values by treatment (Family*Inoculation, Irrigation*Inoculation)

Measurement	ANOVA F-Test Values and P-Values by Treatment Combination					
	Family*Inoculation			Irrigation*Inoculation		
	df	F-value	*P*-Value	df	F-value	*P*-Value
Seedling Volume Change	12	0.75	0.699	8	1.48	0.158
Dry Matter Total	12	1.17	0.302	8	1.07	0.383
Needles	12	1.15	0.318	8	1.28	0.254
Shoots	12	0.98	0.465	8	0.98	0.453
Aboveground (Ne+Sh)	12	1.21	0.277	8	1.25	0.268
Coarse Roots	12	0.78	0.675	8	0.54	0.828
Fine Roots	12	0.82	0.631	8	1.25	0.268
Belowground (Cr+Fr)	12	0.77	0.685	8	0.84	0.568
SPAD	12	1.33	0.195	8	1.33	0.225
Lesion Length	9	4.48	< 0.0001***	6	0.77	0.591
Lesion Volume	9	2.07	0.029*	6	2.44	0.024*
Lesion Length/ Seedling Height	9	2.57	0.006*	6	0.27	0.950
Lesion Volume/ Seedling Volume	9	1.14	0.332	6	1.04	0.395
Mid-Day Wet Water Potential	12	1.43	0.154	8	2.68	0.007*
Mid-Day Dry Water Potential	12	1.24	0.253	8	0.53	0.832
Pre-Dawn Wet Water Potential	12	0.5	0.913	8	1.01	0.428
Pre-Dawn Dry Water Potential	n/a	n/a	n/a	n/a	n/a	n/a

* denotes $P < 0.05$; ** denotes $P < 0.001$; *** denotes $P < 0.0001$. F(df, n) denotes the degrees of freedom (df) and sample size (n) of the ANOVA F-Test. “n/a” denotes data were not analyzed.

Table 3.3d. ANOVA F-Test Values and *P*-Values by treatment

Measurement	**ANOVA F-Test Values and P-Values by Treatment Combination**		
	Family*Irrigation* Inoculation		
	df	**F-value**	***P*-Value**
Seedling Volume Change	24	0.69	0.865
Dry Matter_Total	24	1.41	0.097
Needles	24	1.29	0.171
Shoots	24	1.45	0.082
Aboveground (Ne+Sh)	24	1.44	0.087
Coarse Roots	24	1.28	0.179
Fine Roots	24	1.00	0.466
Belowground (Cr+Fr)	24	1.20	0.240
SPAD	24	1.41	0.090
Lesion Length	18	1.17	0.280
Lesion Volume	18	1.11	0.340
Lesion Length/ Seedling Height	18	1.07	0.378
Lesion Volume/ Seedling Volume	18	0.94	0.524
Mid-Day Wet Water Potential	24	0.95	0.527
Mid-Day Dry Water Potential	24	1.10	0.348
Pre-Dawn Wet Water Potential	24	1.17	0.269
Pre-Dawn Dry Water Potential	n/a	n/a	n/a

* denotes $P < 0.05$; ** denotes $P < 0.001$; *** denotes $P < 0.0001$. F(df, n) denotes the degrees of freedom (df) and sample size (n) of the ANOVA F-Test. “n/a” denotes data were not analyzed.

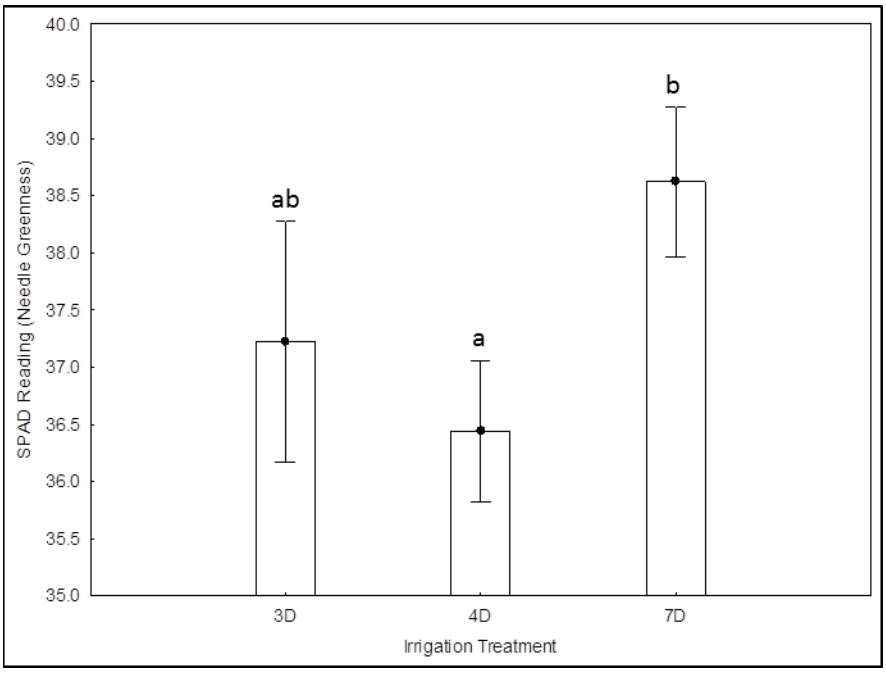

Figure 3.1. Needle greenness by irrigation treatment. Letters are from Tukey pair-wise comparisons. Bars denote 95% confidence intervals. 3D = 3 days.week^{-1} irrigation treatment; 4D = 4 days.week^{-1} irrigation treatment; 7D = 7 days.week^{-1} irrigation treatment.

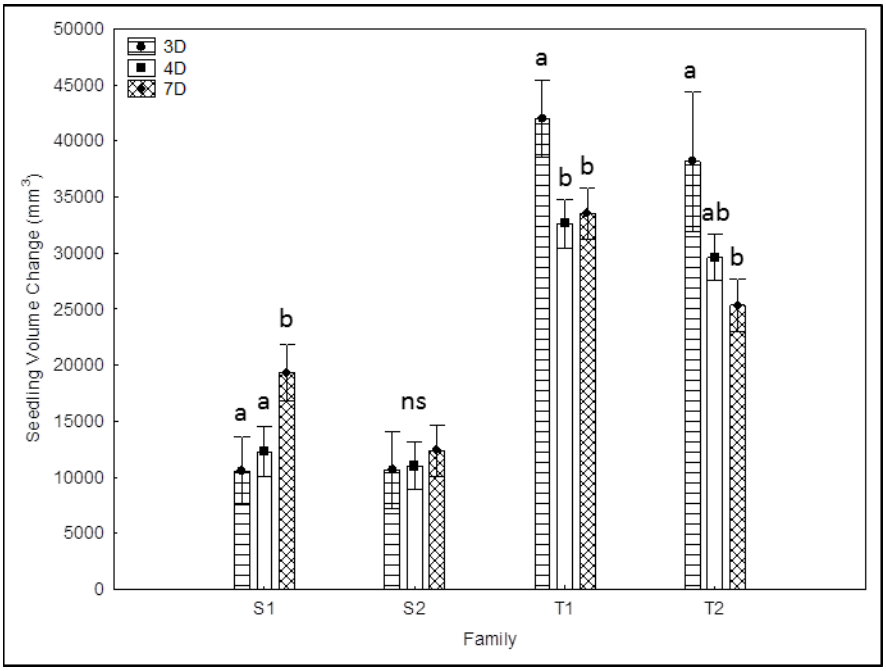

Figure 3.2. Seedling volume change by loblolly pine family by irrigation treatment. Letters are from Tukey pair-wise comparisons and are specific to each family. Bars denote 95% confidence. S1 and S2 denote families selected for susceptibility to root infecting ophiostomatoid fungi. T1 and T2 denote families selected for tolerance to root infecting ophiostomatoid fungi. "ns" denotes "no significance."

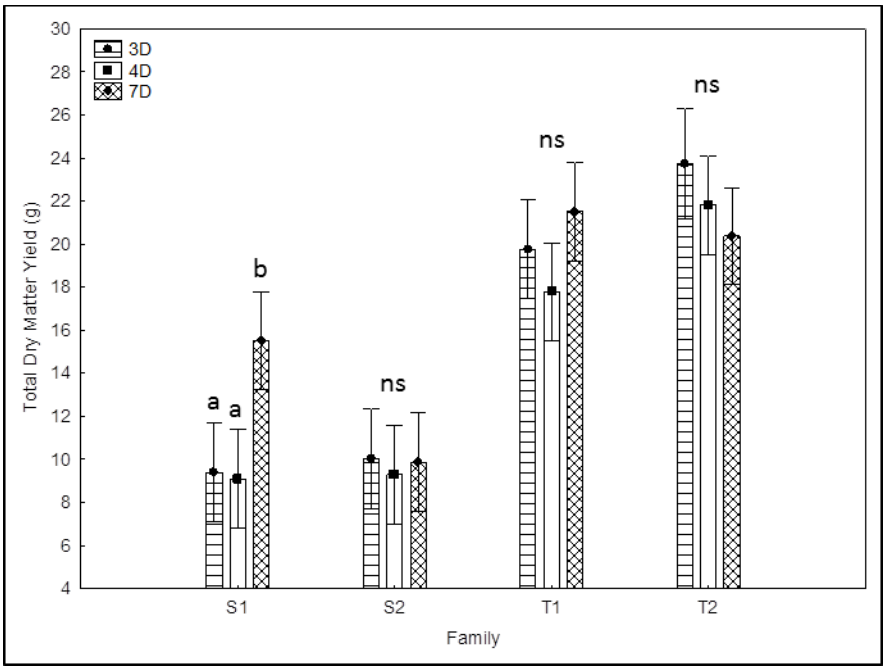

Figure 3.3. Whole plant (total) dry matter yield by loblolly pine family by irrigation treatment. Letters are from Tukey pair-wise comparisons. Bars denote 95% confidence intervals. S1 and S2 denote loblolly pine families selected for their susceptibility to root infecting ophiostomatoid fungi. T1 and T2 denote families selected for their tolerance to root infecting ophiostomatoid fungi.

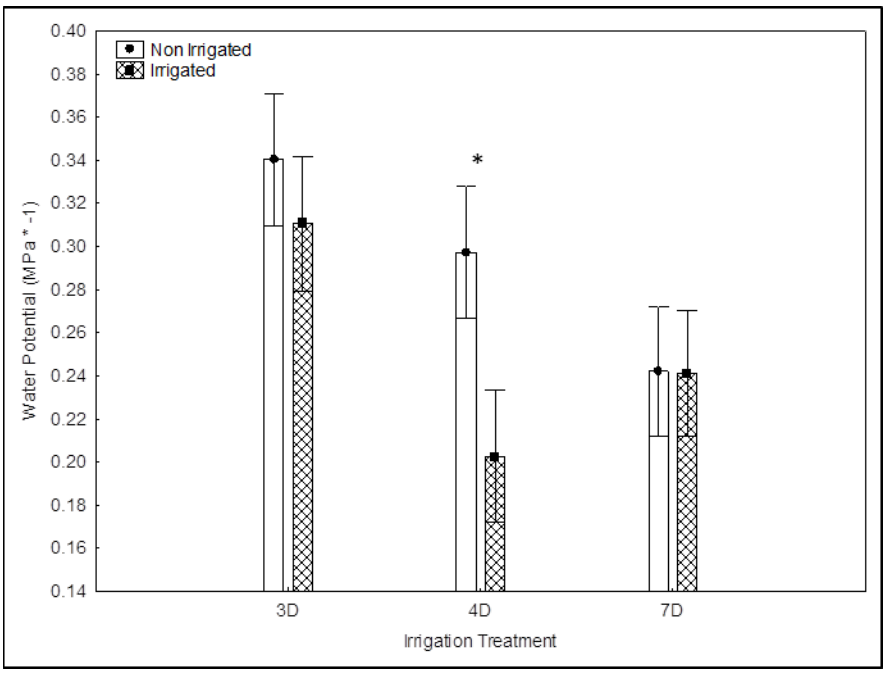

Figure 3.4. Midday water potential (-MPa) by irrigation treatment and day of irrigation. The asterisk denotes a significant difference in water potential. Bars indicated 95% confidence intervals. 3D = 3 days.week^{-1} irrigation treatment; 4D = 4 days.week^{-1} irrigation treatment; 7D = 7 days.week^{-1} irrigation treatment.

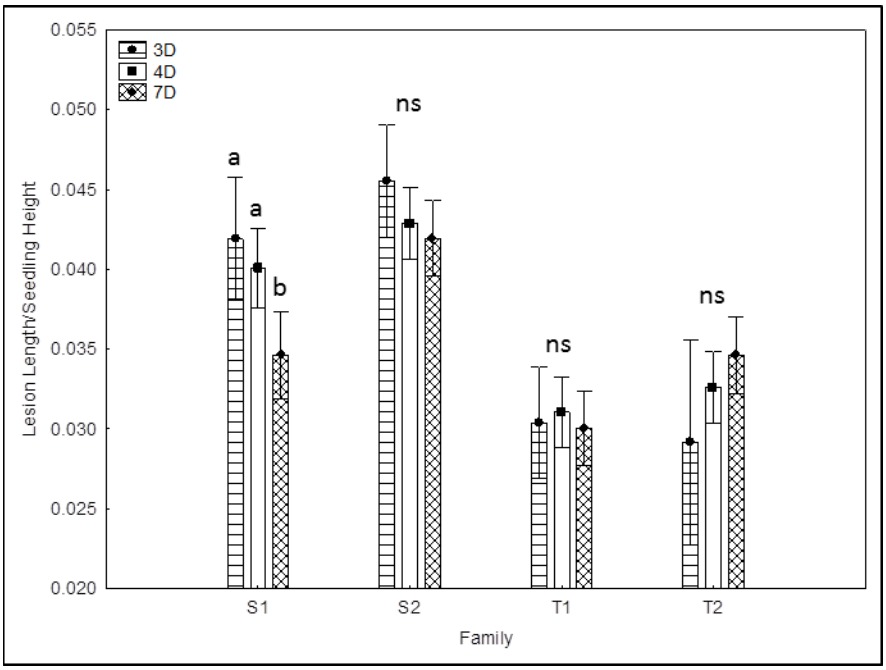

Figure 3.5. Lesion length/seedling height (lesion length ratio) by irrigation treatment and loblolly pine family. Letters indicate post hoc Tukey pair-wise comparisons. “ns” denotes “no significance.” Bars denote 95% confidence intervals.