forests

MDPI

Article

Simulated Summer Rainfall Variability Effects on Loblolly Pine (*Pinus taeda*) Seedling Physiology and Susceptibility to Root-Infecting Ophiostomatoid Fungi

Jeff Chieppa *, Lori Eckhardt and Arthur Chappelka

School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL 36849, USA; eckhalg@auburn.edu (L.E.); chappah@auburn.edu (A.C.)

* Correspondence: jjchieppa@gmail.com; Tel.: +61-0422-851-582

Academic Editors: Matteo Garbelotto and Paolo Gonthier

Received: 26 January 2017; Accepted: 27 March 2017; Published: 30 March 2017

Abstract: Seedlings from four families of loblolly pine (*Pinus taeda* L.) were grown in capped open-top chambers and exposed to three different weekly moisture regimes for 13 weeks. Moisture regimes varied in intensity and frequency of simulated rainfall (irrigation) events; however, the total amounts were comparable. These simulated treatments were chosen to simulate expected changes in rainfall variability associated with climate change. Seedlings were inoculated with two root-infecting ophiostomatoid fungi associated with Southern Pine Decline. We found susceptibility of loblolly pine was not affected by water stress; however, one family that was most sensitive to inoculation was also most sensitive to changes in moisture availability. Many studies have examined the effects of drought (well-watered vs. dry conditions) on pine physiology and host-pathogen interactions but little is known about variability in moisture supply. This study aimed to elucidate the effects of variability in water availability, pathogen inoculation and their interaction on physiology of loblolly pine seedlings.

Keywords: rainfall patterns; *Pinus taeda*; Southern Pine Decline; *Leptographium terebrantis*; *Grosmannia huntii*

1. Introduction

Southern Pine Decline (SPD) is the term attributed to the premature death of *Pinus* spp. in the Southern United States due to a series of biotic and abiotic factors [1–3]. These factors include associated root pathogenic fungi (e.g., *Leptographium terebrantis* Barras and Perry and *Grosmannia huntii* (Rob-Jeffry.) Zipfel, de Beer and Wingfield, and their root-feeding beetle vectors (*Hylastes salebrosus* Eichoff, *H. tenuis* Eichoff, *Hylobius pales* Herbst., and *Pachylobius picivorus* Germar). Predisposing abiotic factors include resource stress (nutrient deficiencies, edaphic factors, and moisture stress), management strategies such as overstocking, mechanical injury and prescribed burning [4]. Studies have shown that when loblolly pine (*P. taeda*) is inoculated with *L. terebrantis*, the fungus can result in the development of lesions in the phloem and resin-soaking in the xylem [5–7]. *Grosmannia huntii*, a non-indigenous species, is a related fungal pathogen and has been reported to be more virulent in young pine seedlings when compared to *L. terebrantis* [7].

In the 1950's, Brown and McDowell [8] observed the decline of mature *P. taeda* stands in Talladega National Forest in Alabama and since then numerous studies have been performed to find causality of the decline as well as detect the phenomena at the landscape level [9–15]. Detection of SPD might be difficult as aboveground symptoms in mature trees (short chlorotic needles, sparse crowns, reduced radial growth, tree morality) occur following root damage and mortality associated with

both associated insects and fungi [3]. Regardless, numerous studies have examined the virulence of root-infecting ophiostomatoid fungi on mature and juvenile families of loblolly pine among other southern *Pinus* species [6,16–18]. Since infection is dependent on the bark beetle vectors, it is important to investigate how predisposing factors (e.g., drought) that lead to root feeding, and thus fungal infection, interact with fungi associated with SPD.

Future climate change scenarios may play a significant role in the predisposing factors associated with SPD. An uncertainty with these potential developments is how much precipitation will occur in the Southern U.S. in the next 50–100 years [19,20]. One of the most important and least studied factors regarding climate change is extremes in climatic variability. For example, in 2007, the worst drought in 100 years occurred in the Southern U.S. and was followed by flooding in 2009 [21]. While changes in the intensity and frequency of summer precipitation may continue in the Southeastern U.S., there is still debate as to the underlying cause [21–23]. 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 [20,24,25].

A concern when considering future precipitation patterns is how forests will respond to altered drying and wetting periods [19,26]. Trees may thrive during wetter periods and experience moisture stress if evaporative losses increase during warmer, drier periods [27]. Droughts can reduce tree vigor and alter insect and pathogen physiology [28]. The effects of precipitation changes are anticipated to be unique based on both the host and pathogen physiology [29–31]. For example, mature forests would likely be tolerant of seasonal variability in rainfall frequency and magnitude [32]. The linkages between tree size and mortality due to changes in precipitation patterns are likely to be size dependent with seedlings and tall trees being most sensitive [33,34]. While tree size is likely important due to physiological constraints when under reduced available moisture [35], climate of origin may be equally important. For example, vegetation communities from more xeric sites may be more sensitive to changes in rainfall magnitude [36], while it is mesic sites that may be more sensitive to changes in frequency of precipitation [37].

The linkages between biotic and abiotic tolerance (cross-tolerance) is a useful tool to help understand how to select appropriate families/genotypes for out planting [31,38–40].In the case of root-infecting ophiostomatoid fungi and loblolly pine seedlings, the role of water regulation is likely important as inoculation can cause resin-soaking in the xylem, which has negative impacts on water movement. Lesions in the phloem can affect carbon transport, which can affect allocation and production of biomass [16,41,42]. The direct effects that water availability can have on physiological traits and productivity is also important [43,44]. Therefore, a suite of response traits (e.g., chlorophyll content, water potential, lesion length) seems appropriate for investigating interactive effects of multiple stressors.

Based on several studies [30,45,46] 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; (3) Climate can change the way the host and pathogen interact, through direct and/or indirect effects. In an assessment of the effect of potential future climate change scenarios for the Southern U.S., Jones et al. [47] stated that changes in variation in water availability are important and require further investigation. Variability in 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 goal of this study, therefore, was to elucidate the interactions of two root-infecting ophiostomatoid fungi (*L. terebrantis* and *G. huntii*) in the presence of climatic conditions similar to those predicted in the next 50 to 100 years in the Southern U.S. More specifically, our main focus was to understand how variability in water availability may affect the outcome of loblolly pine infection with the root-infecting ophiostomatoid fungi. The hypotheses tested include: (1) Loblolly pine will become more susceptible to *L. terebrantis* and *G. huntii*

as variability in water availability increases and (2) Loblolly pine families selected for their tolerance to root-infecting ophiostomatoid fungi would be more tolerant to changes in water availability.

2. Materials and Methods

2.1. 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, USA. The site contained 24 open-top chambers (OTCs), monitoring sheds and a small laboratory. The OTCs were 4.8 m height × 4.5 m diameter aluminum framed structures with fans (1.5 horse-power or 1.1 kw motors) and chamber plastics [48]. Plastic caps were attached to each OTC to exclude ambient rainfall and permit adequate airflow [49].

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.

2.2. Seedlings

Bareroot 1–0 seedlings (sown in March 2013) from four commercially grown loblolly pine families were used for this study (lifted/extracted from the nursery in January 2014). We utilize the term "family" as we did not test genetic distinction between groupings. We also do not use the term ecotype because seedling parents are not from sites with distinct/contrasting ecological characteristics. Based on previous findings, two of these loblolly pine families were considered "tolerant" (T1 and T2) and two "susceptible" (S1 and S2) to root-infecting ophiostomatoid fungi [18,50]. In January 2014, 2700 seedlings (750 per family) were planted in 2.4 liter pots (1 trade gallon) with ProMix BX® peat-based potting mix (Premier Tech, Quebec, Canada). Seedlings were kept in a shade-house and watered daily for 17 weeks until being deployed into the OTCs in May 2014. The seedlings at the commencement of the study were approximately 10 months removed from sowing in the nursery.

2.3. Simulated Rainfall Treatments

To determine the longest duration the saturated potting mix could last without additional water, eight seedlings (two from each family) were placed in a greenhouse at approximately 32 °C (~90 °F). After three days of water being withheld, the potting mix became dried out and therefore, the longest period between simulated rainfall (irrigation) events was set at two days.

Three simulated precipitation treatments were used (3 replicates/treatment) over 13 weeks. The treatments were as follows: (1) 3 days $week^{-1}$ (3D) during the experimental period; (2) 4 days $week^{-1}$ (4D) during the experimental period; and (3) 7 days $week^{-1}$ (7D) during the experimental period. Irrigation nozzles within each OTC were adjusted to ensure an even water distribution and flow rates within and between the chambers. The amount of water distributed was adjusted to ensure 58 minutes of watering resulted in 25.4 mm (1 inch) of precipitation. While the days of watering varied between treatments, each chamber received approximately the same amount of precipitation at the end of each week. Weekly watering values were estimated based on the 30-year (1971–2000) average precipitation for Auburn, AL. Therefore, our target for the water amount from May to August was 97, 103, 149 and 92 mm per month, respectively. Irrigation events occurred three times a day at 09:00, 12:00 and 15:00 hour. In June 2014, a 20% increase was applied to all treatment amounts/time to compensate for higher temperatures and increased airflow in the chamber. Average temperature inside the chambers was about 3–5 °C higher than ambient site temperature [51]. Monitoring throughout June indicated this adjustment approximately offset the increased evaporation of moisture in the chambers.

2.4. Inoculations

Stem inoculations were conducted as described by Nevill et al. [50] 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). The *L. terebrantis* isolate (LOB-R-00-805/MYA-3316) was obtained from a *P. taeda* root exhibiting symptoms characteristic of root disease in Talladega National Forest, Oakmulgee Ranger District, AL. The *G. huntii* isolate (LLP-R-02-100/MYA-3311) was obtained from *Pinus palustris* Mill. root exhibiting symptoms characteristic of root disease in Fort Benning Military Reservation, GA. These isolates were obtained by excavating primary lateral roots, cutting the root into ~1 cm^3 sections, surface sterilizing and plating into 2% malt extract agar (MEA) and MEA with 800 mg/L cycloheximide and 200 mg/L of streptomycin sulfate [3,52]. Isolates were identified using Jacobs and Wingfield [53] by growing them in the dark and using a compound microscope. These isolates have been used in previous studies [3,17,18,54]. Long-term storage of fungi occurred on silica gel [55] at 4° C. *Leptographium terebrantis* is characterized by aerial mycelium but is very general in characteristics of related species. It can be distinguished from other *Leptographium* species by the branching of mycelium. Unlike *L. terebrantis*, *G. huntii* is readily distinguishable by the presence of serpentine-like hyphae and the presence of sexual structures (peithecia) (descriptions from Jacobs and Wingfield [53]). Control seedlings (NW, W and WM) also were plated to determine if contamination had occurred through the presence of *L. terebrantis* and/or *G. huntii*.

To inoculate seedlings, a sterile razor blade was used to cut a 15 mm vertical lesion into the bark (<1 cm depth) 5 cm above the soil line. Plugs on 2% MEA (3 mm) were placed into the wound (a single plug per seedling). Media were either sterile or colonized by cultures of *L. terebrantis* (LT) or *G. huntii* (GH). 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 [18,54].

2.5. Measurements and Harvest

Root collar diameter (RCD) and height measurements were recorded for all seedlings at both the study initiation (February 2014, Table 1) and completion (August 2014) using a digital caliper and meterstick. Seedling volume increment was calculated ($Volume_{Final} - Volume_{Initial} = Volume_{Change}$) to determine overall growth of individual seedlings. The equation $Volume = RCD^2 \times height$ was used to estimate seedling volume/biomass in young pine seedlings (2–3 years of age) [56].

During planting in February, 40 seedlings (extra seedlings not included in the design) from each family (160 total) were destructively harvested and separated into needles (NE), shoot (SH), coarse roots (CR, >2 mm diameter) and fine roots (FR, <2 mm diameter) (Table 1). These components were placed in drying-ovens at 70 °C for 72 h. At the conclusion of the study (August 2014), two seedlings from each treatment combination per chamber were selected for final dry matter seedling biomass. Initial family dry matter averages (the 160 extra seedlings; 40 per family) for each component (needles, shoots etc.) were subtracted from the final dry matter values to estimate dry matter yield.

Table 1. Initial family dry matter, root collar diameter (RCD) and height averages and standard deviations (n = 160; 40/family).

Family	Needle (g)	Shoot (g)	Coarse Root (g)	Fine Root (g)	RCD (mm)	Height (mm)
S1	8.35 ± 0.87	7.54 ± 0.53	7.15 ± 0.40	6.46 ± 0.26	4.10 ± 0.72	263.06 ± 41.65
S2	8.50 ± 0.89	7.76 ± 0.62	6.73 ± 0.31	6.28 ± 0.10	4.18 ± 0.89	267.08 ± 28.12
T1	11.36 ± 1.94	8.90 ± 1.22	8.03 ± 0.99	6.58 ± 0.38	5.81 ± 1.47	283.04 ± 42.53
T2	9.22 ± 1.16	7.80 ± 0.56	6.78 ± 0.34	6.31 ± 0.13	6.29 ± 1.52	279.69 ± 35.13

S1, S2 denote loblolly pine families selected for their susceptibility to root-infecting ophiostomatoid fungi while T1, T2 denotes families selected for their tolerance.

Eleven seedlings from each treatment combination, from each of the nine OTCs, were examined for relative leaf chlorophyll (or needle greenness) using a SPAD-502 chlorophyll meter (Spectrum Tech. Inc., Plainfield, IL, USA) during the final harvest (August 2014). Needles from the first 2013 flush (previous year) were selected as they had reached physiological maturity [57]. A group of 5 to 7 needles were used for measurements. Two measurements were taken on separate parts of a plant and averaged. The same seedlings were measured for lesion characteristics. Seedlings were cut at the soil line, and stems were placed in plastic bins filled with FastGreen stain (FastGreen FCF; Sigma Chemical Co., St. Louis, MO, USA) as described by Singh et al. [18]. After 72 h, stems were removed, and the lesion length and occlusion length were measured. Lesions are the portion of the phloem colonized by *L. terebrantis* and *G. huntii*. The occlusion is the portion of the xylem that does not conduct water due to resin-soaking (thus the use of FastGreen stain). Lesion/occlusion length is presented on a per height basis (lesion length ratio) to standardize for plant growth (e.g., a 50 mm long lesion would be functionally different between plants with heights of 100 cm and 300 cm). Two 5 mm cross-sections 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 [18].

The remaining two seedlings from each combination treatment per chamber were sampled for water potential using a Scholander pressure bomb (PMS Instrument Company, Albany, OR, USA) during the final week of the experimental period. Five cm of a randomly selected lateral branch for each seedling was excised and sampled as described by Kaufmann [58]. Predawn water potential sampling occurred between 03:00 and 05:00 hour. Seedlings were sampled on watered and non-watered days. For example, those irrigated 4 day/week were measured following a day of watering and the following day after a day with no watering (herein referred to wet and dry). Seedlings irrigated daily (7D) were measured twice and randomly assigned the treatment 'watered' and 'non-watered'. This allows for the experimental design to be balanced (important for statistical analysis used, see Section 2.6) in addition to determine if seedling water potential values were significantly affected by the rainfall treatments themselves.

2.6. Data Analysis

The experimental design was a split-split-split plot with replicates at all levels: three simulated rainfall treatments replicated 3 times (9 total chambers), 4 loblolly pine families and 5 inoculation treatments produced 60 treatment combinations. Each treatment combination was replicated 15 times per chamber at the initiation of the study. Those seedlings where fungi were not recovered from re-isolations were excluded from the analysis as changes in measured characteristics cannot be attributed to the presence of the inoculated fungi. All statistical analyses were conducted using SAS (Version 9.3, SAS Institute, Inc., Cary, NC, USA) and STATISTICA (Statsoft, Inc., Tulsa, OK, USA). ANOVA *F*-test procedures followed by post hoc Tukey (Honest Significant Difference) procedures were used to determine individual treatment effects. ANOVA assumptions were verified (checked for normality) using both Kolmogorov-Smirnov and Lilliefors tests. Homogeneity of variance was inspected visually in addition to using both Levene's and Bartlett's tests [59,60]. Alpha was set at 0.05.

3. Results

3.1. Overall Results

An overview of significant interactions is presented in Table 2, including transformations utilized and number of plants used for the analysis. An overview of significant interacts for water potential data is presented in Table 3 ($p < 0.05$).

Table 2. Results of ANOVA *F*-tests.

Measurement	Transformation	*n*	Treatments/Combinations						
			Rain	Family	Inoculation	Rain × Fam	Rain × Inoc	Fam × Inoc	Rain × Fam × Inoc
SPAD	Square root	1561	***	NS	NS	NS	NS	NS	NS
Seedling volume increment	Log_{10}	2043	*	***	NS	***	NS	NS	NS
Total Dry Matter Yield	Log_{10}	298	**	***	NS	**	NS	NS	NS
Needle DMY	Square root	298	NS	***	**	***	NS	NS	NS
Shoot DMY	Square root	298	**	***	NS	*	NS	NS	NS
Coarse Root DMY	Square root	298	***	***	NS	*	NS	NS	NS
Fine Root DMY	Square root	298	***	***	**	NS	NS	NS	NS
Lesion Length/Seedling Height	Log_{10}	1194	NS	***	***	**	NS	**	NS
Occlusion Length/Seedling	Log_{10}	1161	NS	***	***	*	NS	**	NS

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$; NS: not significant; Fam: Family, Inoc: Inoculation.

Table 3. Results of ANOVA *F*-tests for water potential data.

Treatments/Combinations	Predawn Water Potential
	***n* = 787**
	Transformation: Square Root
Rain	****
Family	NS
Inoculation	NS
Dry-wet (DW)	*
Rain × Fam	***
Rain × Inoc	*
Rain × DW	NS
Fam × Inoc	NS
Fam × DW	NS
Inoc × DW	NS
Rain × Fam × Inoc	**
Rain × Fam × DW	NS
Rain × Inoc × DW	NS
Fam × Inoc × DW	NS
Rain × Fam × Inoc × DW	NS

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$; NS: not significant; Fam: Family, Inoc: Inoculation.

Tukey pair-wise comparisons are used to denote significant differences during post-hoc analysis. Letters that are the same represent no significant difference, while those that are different represent a significant difference.

3.2. Leaf Chlorophyll/Needle Greeness

Leaf chlorophyll and needle greenness were affected significantly by the rainfall treatment only. Seedlings irrigated 7D (38.2 ± 0.7) were significantly different from those irrigated 4D (35.9 ± 0.3); however, those irrigated 3D (36.8 ± 1.2) were not different from either 7D and 3D seedlings.

3.3. Seedling Volume Increment

Seedling volume increment (mm^3) was affected significantly by rainfall, family and the interaction between rainfall and family. Within the rainfall × family interaction, S1 grew significantly more when watered 7D (Table 4). The remaining families were not significantly affected.

Table 4. Summary of seedling volume increment (mm^3) by rainfall and family.

Family	3D			4D			7D		
	Mean (mm^3)	95% Confidence Interval	Tukey Pair-Wise	Mean (mm^3)	95% Confidence Interval	Tukey Pair-Wise	Mean (mm^3)	95% Confidence Interval	Tukey Pair-Wise
S1	1243	±136	A	1377	±115	A	1854	±171	B
S2	1013	±123	A	1166	±90	A	1273	±105	A
T1	3144	±385	A	2729	±220	A	2877	±239	A
T2	3152	±668	A	2424	±184	A	2261	±191	A

Tukey pair-wise comparisons are within families.

3.4. Dry Matter Yield (DMY)

Total DMY was significantly affected by rainfall, family and the interaction between rainfall and family. For the rainfall × family interaction, S1 was the only family to be significantly affected by the rainfall treatment, where it produced more dry matter when watered 7D (Table 5).

Table 5. Summary of seedling total dry matter yield (g) by rainfall and family.

Family	3D			4D			7D		
	Mean (g)	95% Confidence Interval	Tukey Pair-Wise	Mean (g)	95% Confidence Interval	Tukey Pair-Wise	Mean (g)	95% Confidence Interval	Tukey Pair-Wise
S1	8.93	±1.22	A	8.37	±1.14	A	13.43	±1.83	B
S2	8.88	±1.23	A	8.29	±1.13	A	9.17	±1.25	A
T1	17.40	±2.37	A	17.00	±2.32	A	19.95	±2.72	A
T2	23.48	±3.84	A	20.76	±2.83	A	19.20	±2.58	A

Tukey pair-wise comparisons are within families.

Needle DMY was significantly affected by family, inoculation, and rainfall × family. Needle DMY increased significantly when seedlings were inoculated with GH (8.38 ± 0.72) compared to NW and W seedlings (6.73 ± 0.72 and 6.58 ± 0.73, respectively). Seedlings with WM and LT treatment (8.01 ± 0.72 and 7.56 ± 0.72, respectively) were not different from any other treatment. Seedling needle DMY for S1 was significantly affected by the irrigation treatments, with greater needle DMY when watered 7D (Table 6). The remaining families were not significantly affected.

Table 6. Summary of seedling needle dry matter yield (DMY, g) by rainfall and family.

Family	3D			4D			7D		
	Mean (g)	95% Confidence Interval	Tukey Pair-Wise	Mean (g)	95% Confidence Interval	Tukey Pair-Wise	Mean (g)	95% Confidence Interval	Tukey Pair-Wise
S1	4.46	±0.79	A	4.31	±0.77	A	7.02	±1.00	B
S2	4.76	±0.83	A	4.52	±0.79	A	4.72	±0.81	A
T1	8.80	±1.12	A	7.91	±1.06	A	9.50	±1.17	A
T2	10.98	±1.31	A	10.20	±1.21	A	8.59	±1.09	A

Tukey pair-wise comparisons are within families.

Shoot DMY was significantly affected by rainfall, family, and their interaction. Similar to total DMY, family S1 was the only one affected by rainfall treatment, where it produced greater shoot DM when irrigated 7D (Table 7).

Table 7. Summary of seedling shoot dry matter yield (DMY, g) by rainfall and family.

Family	3D			4D			7D		
	Mean (g)	95% Confidence Interval	Tukey Pair-Wise	Mean (g)	95% Confidence Interval	Tukey Pair-Wise	Mean (g)	95% Confidence Interval	Tukey Pair-Wise
S1	2.73	±0.59	A	2.43	±0.55	A	4.46	±0.76	B
S2	2.53	±0.57	A	2.60	±0.57	A	2.51	±0.56	A
T1	4.88	±0.80	A	4.60	±0.77	A	5.65	±0.86	A
T2	6.14	±0.93	A	5.54	±0.85	A	5.83	±0.86	A

Tukey pair-wise comparisons are within families.

Coarse root DMY was significantly affected by rainfall, family, and their interaction. Similar to total DMY, family S1 was the only one affected by rainfall treatment, where it produced more coarse root material when watered 7D (Table 8).

Table 8. Summary of seedling coarse root dry matter yield (DMY, g) by rainfall and family.

Family	3D			4D			7D		
	Mean (g)	95% Confidence Interval	Tukey Pair-Wise	Mean (g)	95% Confidence Interval	Tukey Pair-Wise	Mean (g)	95% Confidence Interval	Tukey Pair-Wise
S1	1.22	±0.27	A	1.32	±0.28	A	2.01	±0.35	B
S2	1.16	±0.27	A	1.05	±0.25	A	1.15	±0.26	A
T1	2.68	±0.41	A	3.43	±0.46	A	3.66	±0.48	A
T2	3.96	±0.52	A	3.52	±0.47	A	3.71	±0.48	A

Tukey pair-wise comparisons are within families.

Fine root DMY was significantly affected by rainfall, family and inoculation; however, no interactions were found to be significant. Seedlings irrigated 3D and 7D (1.12 ± 0.13 and 1.15 ± 0.13, respectively) produced more fine root DM than those watered 4D (0.86 ± 0.13). Susceptible families (S1 and S2) produced less fine root material than tolerant families (T1 and T2) (Table 9). Seedlings inoculated with GH (1.31 ± 0.18) produced significantly more fine root material than control seedlings (NW: 0.94 ± 0.15, W: 0.89 ± 0.15, and WM: 0.98 ± 0.15). Seedlings inoculated with LT (1.07 ± 0.16) were intermediate and not different from any of the inoculated treatments.

Table 9. Summary of seedling fine root dry matter yield (DMY, g) by family.

Family	Mean (g)	95% Confidence Interval	Tukey Pair-Wise
S1	0.64	0.11	A
S2	0.67	0.11	A
T1	1.54	0.17	B
T2	1.47	0.17	B

Tukey pair-wise comparisons are comparing families.

3.5. Lesions and Occlusions

Fungal re-isolation was 75.4% successful for *L. terebrantis* and 73.4% for *G. huntii.* Control seedlings had a 0% re-isolation rate indicating no contamination had occurred. Lesion length for the controls (W and WM) was not significantly different for any treatment or treatment combination, indicating the wounding process was completed with accuracy. This also indicates no effect from the presence of the media (in WM seedlings). The lesion produced from the wounding process was 14.9 ± 2.0 mm. Lesion length/seedling height for all inoculated seedlings was significantly affected by inoculation (Table 10) indicating successful colonization of seedling tissue by root-infecting ophisotomatoid fungi. Only S1 lesion length/seedling height was affected by rainfall; however, this includes the W and WM controls.

Table 10. Summary of lesion length/seedling height (×100) by family and inoculation treatment.

Inoculation	Family	S1	S2	T1	T2
W	Mean	3.28	3.33	2.59	2.82
	95% Confidence Interval	±0.24	±0.23	±.017	±0.31
	Tukey	A	A	A	A
WM	Mean	3.18	3.40	2.63	2.62
	95% Confidence Interval	±0.23	±0.21	±0.18	±0.24
	Tukey	A	A	A	A
LT	Mean	4.03	5.14	3.17	3.48
	95% Confidence Interval	±0.28	±0.32	±0.19	±0.30
	Tukey	B	B	B	B
GH	Mean	4.37	4.43	3.25	3.39
	95% Confidence Interval	±0.31	±0.30	±0.21	±0.34
	Tukey	B	C	B	B

Tukey pair-wise comparisons are within family.

Occlusion length/seedling height was significantly affected by family, inoculation, rainfall × family, and family × inoculation. The rainfall × family post-hoc analysis yielded results that indicated significant differences between families (not within) and therefore do not warrant further examination. The family × inoculation results show that W and WM seedlings were not different for any family (Table 11). Seedlings inoculated with GH had a lower occlusion length/seedling height than those inoculated with LT for each family with the exception of S1, where they were not significantly different.

Table 11. Summary of occlusion length/seedling height by family and inoculation treatment.

Inoculation	Family	S1	S2	T1	T2
W	Mean	1.52	1.51	1.43	1.45
	95% Confidence Interval	±0.03	±0.03	±0.03	±0.05
	Tukey	A	A	A	A
WM	Mean	1.50	1.53	1.42	1.42
	95% Confidence Interval	±0.03	±0.03	±0.03	±0.04
	Tukey	A	A	A	A
LT	Mean	1.76	1.89	1.66	1.67
	95% Confidence Interval	±0.04	±0.03	±0.03	±0.04
	Tukey	B	B	B	B
GH	Mean	1.69	1.69	1.57	1.55
	95% Confidence Interval	±0.04	±0.03	±0.03	±0.05
	Tukey	B	C	C	C

Tukey pair-wise comparisons are within family.

3.6. Predawn Water Potential

Water potential (megapascals) was affected by many treatments and treatment combinations. Since the rainfall × family × inoculation was significant, the main effects of each of these treatments (and their two-way interactions) cannot be analyzed. The rainfall × family × inoculation post-hoc analysis results showed that significant differences are not comparable (e.g., comparing S1-NW-7D to T2-GH-3D seedlings). There was a significant difference between measurements taken on days following watering (wet: 0.129 ± 0.009) and those taken on days following watering being withheld (dry: 0.146 ± 0.010).

4. Discussion

To our knowledge, no studies have utilized fluctuating moisture availability to mimic predicted changes in rainfall periodicity in tandem with root-pathogen inoculations. The variability in simulated rainfall patterns in the OTCs 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. We did observe a trend that families chosen for their tolerance to root-infecting ophiostomatoid fungi tended to have greater growth rates and produce more dry matter. Results from this study should be reviewed with caution as the experimental duration (13 weeks) was short, while the impact of changed precipitation patterns could have effects over longer periods. In addition, root-infecting ophiostomatoid fungi associated with SPD affect mature trees; however, the use of seedlings to screen families for tolerance has been useful in predicting the response of mature trees (Eckhardt et al., 2004).

Water stress has been found to result in a decrease in net photosynthesis in loblolly pine [61], which is accompanied by a decrease in transpiration rate [62]. Seiler and Johnson [63] found evidence that water stress conditioning allowed loblolly pine seedlings to photosynthesize at lower water potentials than usual. This may explain why, in our study, seedlings watered 3D were not different from those watered 4D or 7D. The confidence interval for SPAD measurements for seedlings watered 3D was nearly twice that of the other treatments, which could indicate some had begun to become acclimated to the simulated rainfall treatment. Overall, needle greenness was not affected by any other treatment. This could indicate that needle greenness, or more broadly photosynthesis, is unrelated to susceptibility of loblolly pine to root-infecting ophisotomatoid fungi.

Loblolly pine has been shown to have reduced growth when exposed to moisture stress [64,65] and the degree of response is linked to seed source location [63]. In our study, only one susceptible family had reduced growth (volume growth and dry matter yield) when exposed to altered rainfall amounts. In general, tolerant families produce more volume and biomass compared to susceptible families. This could indicate that families that have greater relative growth rates are less susceptible to root-infecting ophiostomatoid fungi. In this study, we found the wounding process to be of particular importance. This could indicate tolerant families allocate photosynthates differently than susceptible families. Future research should investigate resource allocation of photosynthates to structural and chemical properties of wood. We did observe changes in biomass allocation (needles, roots, etc.) with inoculation treatment; however, no strong pattern emerged. Given the short duration of the study, a pattern may have been difficult to detect, and we recommend a longer experimental duration to determine if trends exist regarding biomass allocation and inoculation with fungi associated with SPD. Changes in allocation of biomass can have effects on acquisition (e.g., root length) and storage (e.g., leaf water holding capacity) of water, which in turn can affect plant response to drought. Numerous studies have examined the effects of precipitation magnitude (flooding or drought) on plants and fungal pathogen interactions. These studies usually compare a sufficiently watered control and a reduced water treatment [16,63,64,66,67]. While these findings provide insight into host plant responses to periods of reduced moisture availability, less is known about the impact that variability in moisture availability will have on host-pathogen interactions. Some reports indicate that these alterations result in decreased productivity of loblolly pine [65], but widespread evidence is scarce and lacking with respect to fungal pathogens. In a previous investigation using the same families/genotypes [54], it was observed that inoculation with root-infecting ophiostomatoid fungi increased water stress when compared to non-inoculated control seedlings. In this study, no pattern in water potential emerged with respect to inoculation and the inoculation $\times$ rainfall interaction. Overall, the rainfall treatment had a significant effect, indicating the treatments were affecting seedling water relations. Future analysis should utilize a more in-depth analysis of hydraulic features of loblolly pine seedlings.

Gooheen et al. [66] found increased susceptibility of *P. ponderosa* (Laws.) to a root-infecting ophiostomatoid fungi with wetter soil; however, seedlings were inoculated below the root crown. In that study, the success of the fungal pathogen seemed to be driven by increased moisture; however,

these results could be caused by the effect of moisture on the pathogen. In our study, inoculation occurred above the soil line [50], which does not directly increase moisture access of the pathogen. Croisé et al. [67] found severe drought stress increased susceptibility of *P. sylvestris* (L.) to *L. wingfieldii* (Morelet) as well as a significant decrease in hydraulic conductivity. Our results do not indicate an increase in susceptibility of loblolly pine or a significant change in plant water status. Working with loblolly pine, Meier et al. [64] found decreases in soil moisture led to decreases in available carbohydrates for both above- and belowground biomass. We observed similar results in that decreased moisture availability decreased total biomass yield. We found that this response was not ubiquitous but was rather specific to family.

5. 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 that was sensitive to changes in moisture also had a larger lesion or wound when watered 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 one family of loblolly pine. We observed that families chosen for their tolerance to root-infecting ophiostomatoid fungi were tolerant to the rainfall treatment itself. Therefore, we fail to reject the hypothesis that families selected for their tolerance would also be more tolerant to changes in water availability. The results from this study indicate that the topic of linkages between tolerance to both drought and root-infecting ophiostomatoid fungi warrants further investigation. The authors recommend utilizing more families of loblolly pine in addition to experiments of longer duration, particularly with trees of varying age.

Acknowledgments: The authors would like to give special thanks to Scott Enebak for his guidance and assistance in preparing and reviewing the manuscript, Ryan Nadel for reviewing the manuscript and offering statistical advice, and Efrem Robbins for assistance with maintaining the field site and data collection. In addition, we would like to acknowledge the critiques of two anonymous reviewers of a previous version of this manuscript. Partial funding was provided by an Alabama Agricultural Experiment Station Internal grant award (AAES-Hatch-Multi-State-04) to Chappelka (PI) and the Forest Health Cooperative at Auburn University.

Author Contributions: All authors conceived and designed the experiments; J.C. performed the experiments; J.C. analyzed the data; L.E. and A.C. contributed reagents/materials/analysis tools; J.C. wrote the paper. A.C. provided the field site and technician to maintain the equipment.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Harrington, T.C.; Cobb, F.W., Jr. Pathogenicity of *Leptographium* and *Verticicladiella* spp. isolated from roots of Western North American conifers. *Phytopathology* **1983**, *73*, 596–599. [CrossRef]
2. Otrosina, W.J.; Hess, N.J.; Zarnoch, S.J.; Perry, T.J.; Jones, J.P. Blue-stain fungi associated with roots of Southern pine trees attacked by the Southern Pine Beetle, *Dendroctonus frontalis*. *Plant Dis.* **1997**, *81*, 942–945. [CrossRef]
3. Eckhardt, L.G.; Weber, A.M.; Menard, R.D.; Jones, J.P.; Hess, N.J. Insect-fungal complex associated with Loblolly Pine Decline in central Alabama. *For. Sci.* **2007**, *53*, 84–92.
4. Eckhardt, L.G.; Sayer, M.A.S.; Imm, D. State of Pine Decline in the Southeastern United States. *South. J. Appl. For.* **2010**, *34*, 138–141.
5. Wingfield, M.J. Association of *Verticicladiella procera* and *Leptographium terebrantis* with Insects in the Lake States. *Can. J. For. Res.* **1983**, *13*, 1238–1245. [CrossRef]
6. Eckhardt, L.G.; Jones, J.P.; Klepzig, K.D. Pathogenicity of *Leptographium* species associated with Loblolly Pine Decline. *Plant Dis.* **2004**, *88*, 1174–1178. [CrossRef]

7. Matusick, G.; Eckhardt, L.G. Variation in virulence among four root-inhabiting ophiostomatoid fungi on *Pinus taeda* L., *P. palustris* Mill, and *P. elliottii* Engelm. seedlings. *Can. J. Plant Pathol.* **2010**, *32*, 361–367. [CrossRef]
8. Brown, H.D.; McDowell, W.E. *Status of Loblolly Pine Die-Off on the Oakmulgee District, Talladega National Forest, Alabama*; USDA Forest Service: Pineville, LA, USA, 1968; Volume 28.
9. Brown, H.D.; Peacher, P.H.; Wallace, H.N. *Status of Loblolly Pine Die-Off on the Oakmulgee District, Talladega National Forest, Alabama*; USDA Forest Service: Pineville, LA, USA, 1969; Volume 9.
10. Roth, E.R.; Peacher, P.H. *Alabama Loblolly Pine Die-Off Evaluation*; USDA Forest Service: Pineville, LA, USA, 1971; Volume 9.
11. Hess, N.J.; Otrosina, W.J.; Jones, J.P.; Goddard, A.J.; Walkinshaw, C.H. Reassessment of Loblolly Pine Decline on the Oakmulgee Ranger District, Talladega National Forest, Alabama. In Proceedings of the Tenth Biennial Southern Silvicultural Research Conference, Shreveport, LA, USA, 16–18 February 1999; pp. 560–564.
12. Hess, N.J.; Otrosina, W.J.; Carter, E.A.; Steinman, J.R.; Jones, J.P.; Eckhardt, L.G.; Weber, A.M.; Walkinshaw, C.H. Assessment of loblolly pine decline in central Alabama. In Proceedings of the Eleventh Southern Silvicutural Research Conference, Asheville, NC, USA, 20–22 March 2002; pp. 558–564.
13. Eckhardt, L.G.; Menard, R.D. Topographic features associated with loblolly pine decline in Central Alabama. *For. Ecol. Manag.* **2008**, *255*, 1735–1739. [CrossRef]
14. Zeng, Y.; Kidd, K.R.; Eckhardt, L.G. The effect of thinning and clear-cut on changes in the relative abundance of root-feeding beetle (Coleoptera: Curculionidae) in *Pinus taeda* plantations in central Alabama and Georgia. *Pest Manag. Sci.* **2017**, *70*, 915–921. [CrossRef] [PubMed]
15. Coyle, D.R.; Klepzig, K.D.; Koch, F.H.; Morris, L.A.; Nowak, J.T.; Oak, S.W.; Otrosina, W.J.; Smith, W.D.; Gandhi, K.J. A review of southern pine decline in North America. *For. Ecol. Manag.* **2015**, *349*, 134–148. [CrossRef]
16. Matusick, G.; Eckhardt, L.G.; Enebak, S.A. Virulence of *Leptographium serpens* on Longleaf Pine Seedlings under Varying Soil Moisture Regimes. *Plant Dis.* **2008**, *92*, 1574–1576. [CrossRef]
17. Matusick, G.G.; Somers, L.; Eckhardt, L.G. Root lesions in large loblolly pine (*Pinus taeda* L.) following inoculation with four root-inhabiting ophiostomatoid fungi. *For. Pathol.* **2012**, *42*, 37–43. [CrossRef]
18. Singh, A.; Anderson, D.; Eckhardt, L.G. Variation in resistance of Loblolly pine (*Pinus taeda* L.) families against *Leptographium* and *Grosmannia* root fungi. *For. Pathol.* **2014**, *44*, 293–298.
19. MacCracken, M.; Barron, E.; Easterling, D.; Felzer, B.; Karl, T. *Scenarios for Climate Variability and Change: The Potential Consequences of Climate Variability and Change for the United States*; US Global Change Research Program, National Science Foundation: Washington, DC, USA, 2000.
20. IPCC. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2013; p. 1535.
21. Wang, H.; Fu, R.; Kumar, A.; Li, W. Intensification of summer rainfall variability in the Southeastern United States during recent decades. *J. Hydrometeorol.* **2010**, *11*, 1007–1018. [CrossRef]
22. Li, W.; Li, L.; Fu, R.; Deng, Y.; Wang, H. Changes to the North Atlantic Subtropical High and its role in the intensification of summer rainfall variability in the Southeastern United States. *J. Clim.* **2011**, *24*, 1499–1506. [CrossRef]
23. Seager, R.; Tzanova, A.; Nakamura, J. Drought in the Southeastern United States: Causes, variability over the last millennium, and the potential for future hydroclimate change. *J. Clim.* **2009**, *22*, 5021–5045. [CrossRef]
24. Kunkel, K.E.; Karl, T.R.; Brooks, H.; Kossin, J.; Lawrimore, J.H.; Arndt, D.; Bosart, L. Monitoring and understanding trends in extreme storms: State of knowledge. *Bull. Am. Meteorol. Soc.* **2013**, *94*, 499–514. [CrossRef]
25. Muschinski, T.; Katz, J.I. Trends in hourly rainfall statistics in the United States under a warming climate. *Nat. Clim. Chang.* **2013**, *3*, 577–580. [CrossRef]
26. Hanson, P.J.; Weltzin, J.F. Drought disturbance from climate change: Response of United States forests. *Sci. Total Environ.* **2000**, *262*, 2005–2220. [CrossRef]
27. Neilson, R.P.; Drapek, R.J. Potentially complex biosphere responses to transient global warming. *Glob. Chang. Biol.* **1998**, *4*, 505–521. [CrossRef]

28. Dale, V.H.; Joyce, L.A.; McNulty, S.; Neilson, R.P.; Ayres, M.P.; Flannigan, M.D.; Hanson, P.J. Climate change and forest disturbances: Climate change can affect forests by altering the frequency, intensity, duration, and timing of fire, drought, introduced species, insect and pathogen outbreaks, hurricanes, windstorms, ice storms or landslides. *BioScience* **2001**, *51*, 723–734. [CrossRef]
29. Rouault, G.; Candau, J.; Lieutier, F.; Nageleisen, L.; Martin, J.; Warzée, N. Effects of drought and heat on forest insect populations in relation to the 2003 drought in Western Europe. *Ann. For. Sci.* **2006**, *63*, 613–624. [CrossRef]
30. Sturrock, R.N.; Frankel, S.J.; Brown, A.V.; Hennon, P.E.; Kliejunas, J.T.; Lewis, K.J.; Worrall, J.J.; Woods, A.J. Climate change and forest diseases. *Plant Pathol.* **2011**, *60*, 133–149. [CrossRef]
31. Desprez-Loustau, M.; Marçais, B.; Nageleisen, L.; Piou, D.; Vannini, A. Interactive effects of drought and pathogens in forest trees. *Ann. For. Sci.* **2006**, *63*, 597–612. [CrossRef]
32. Gonthier, P.; Giordana, L.; Nicolotti, G. Further observations of sudden diebacks of Scots pine in the European Alps. *For. Chron.* **2010**, *86*, 110–117. [CrossRef]
33. Mueller, R.; Scrudder, C.; Porter, M.; Trotter, R.; Gehring, C.; Whitman, T. Differential tree mortality in response to severe drought: Evidence from long-term vegetation shifts. *J. Ecol.* **2005**, *93*, 1085–1093. [CrossRef]
34. McDowell, N.; Pockman, W.T.; Allen, C.D.; Breshears, D.D.; Cobb, N.; Kolb, T.; Plaut, J.; Sperry, J.; West, A.; William, D.G.; et al. Mechanisms of plant survival and mortality during drought: Why do some plants survive while other succumb to drought? *New Phytol.* **2008**, *178*, 719–739. [CrossRef] [PubMed]
35. Hanson, J.P.; Todd, D.E.; Amthor, J.S. A six-year study of sapling and large-tree growth and mortality responses to natural and induced variability in precipitation and throughfall. *Tree Physiol.* **2001**, *21*, 345–358. [CrossRef] [PubMed]
36. Huxman, T.E.; Smith, M.D.; Fay, P.A.; Knapp, A.K.; Shaw, M.R.; Loik, M.E.; Smith, S.D.; Tissue, D.T.; Zack, J.C.; Weltzin, J.F.; et al. Convergence across biomes to a common rain-use efficiency. *Nature* **2004**, *429*, 651–654. [CrossRef] [PubMed]
37. Knapp, A.K.; Beier, C.; Briske, D.D.; Classen, A.T.; Luo, Y.; Reichstein, M.; Smith, M.D.; Smith, S.D.; Bell, J.E.; Fay, P.A.; et al. Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience* **2008**, *58*, 811–821. [CrossRef]
38. Vallelian-Bindschedler, L.; Schqeizer, P.; Mosinger, E.; Metraux, J.P. Heat-induced resistance in barley to powdery mildew (*Blumeria graminisf.* sp. *Hordei*) is associated with a burst of active oxygen species. *Physiol. Mol. Plant Pathol.* **1998**, *52*, 185–199.
39. Bowler, C.; Fluhr, R. The role of calcium and activated oxygen as signals for controlling cross-tolerance. *Trends Plant Sci.* **2000**, *5*, 241–246. [CrossRef]
40. Bansal, S.; Hallsby, G.; Löfvenius, M.O.; Nilsson, M.-C. Synergistic, additive and antagonistic impacts of drought and herbivory on *Pinus sylvestrisis*: Leaf, tissue and whole-plant responses and recovery. *Tree Physiol.* **2013**, *33*, 451–463. [CrossRef] [PubMed]
41. Horner, W.E.; Alexander, S.A. Permeability of asymptomatic, resin-soaked and *Verticicladiella procerai*-black-stained pine sapwood. *Phytopathology* **1985**, *75*, 1368.
42. Joseph, G.; Kelsey, R.G.; Thies, W.G. Hydraulic conductivity in roots of ponderosa pine infected with black-stain (*Leptographium wageneri*) or annosus (*Heterobasidion annosum*) root disease. *Tree Physiol.* **1998**, *18*, 333–339. [CrossRef] [PubMed]
43. Contry, J.P.; Smillie, R.M.; Küppers, M.; Bevege, D.I.; Barlow, E.W. Chlorophyll a fluorescence and photosynthetic and growth responses of *Pinus radiate* to phosphorus deficiency, drought stress, and high CO_2. *Plant Physiol.* **1986**, *81*, 423–429.
44. Cregg, B.M.; Zhang, J.W. Physiology and morphology of *Pinus sylvestris* seedlings from diverse sources under cyclic drought stress. *For. Ecol. Manag.* **2001**, *154*, 131–139. [CrossRef]
45. Garrett, K.A.; Dendy, S.P.; Frank, E.E.; Rouse, M.N.; Travers, S.E. Climate change effects on plant disease: Genomes to ecosystems. *Annu. Rev. Phytopathol.* **2006**, *44*, 489–509. [CrossRef] [PubMed]
46. Manning, W.J.; von Tiedemann, A. Climate change: Potential effects of increased atmospheric Carbon dioxide (CO_2), ozone (O_3), and ultraviolet-B (UV-B) radiation on plant diseases. *Environ. Pollut.* **1995**, *88*, 219–245. [CrossRef]

47. Jones, J.; Hatch, U.; Murray, B.; Jagtap, S.; Cruise, J.; Yields, A.C. Potential consequences of climate variability and change for the Southeastern United States. In *Climate Change Impacts on the United States-Foundation Report: The Potential Consequences of Climate Variability and Change*; Cambridge University Press: Cambridge, MA, USA, 2001; Volume 137.
48. Gilliland, N.J.; Chappelka, A.H.; Muntifering, R.B.; Booker, F.L.; Ditchkoff, S.S. Digestive utilization of ozone-exposed forage by rabbits (*Oryctolagus cuniculus*). *Environ. Pollut.* **2012**, *163*, 281–286. [CrossRef] [PubMed]
49. Heagle, A.S.; Philbeck, R.B.; Ferrell, R.E.; Heck, W.W. Design and performance of a large, field exposure chamber to measure effects of air quality on plants. *J. Environ. Qual.* **1989**, *18*, 361–368. [CrossRef]
50. Nevill, R.J.; Kelley, W.D.; Hess, N.J.; Perry, T.J. Pathogenicity to Loblolly pines of fungi recovered from trees attacked by Southern Pine Beetles. *South. J. Appl. For.* **1995**, *19*, 78–83.
51. Chappelka, A.; School of Forestry and Wildlife Sciences, Auburn University. Personal communication, 2014.
52. Hicks, B.R.; Cobb, F.W., Jr.; Gersper, P.L. Isolation of *Ceratocystis wageneri* from forest soil with a selective medium. *Phytopathology* **1980**, *70*, 880–883. [CrossRef]
53. Jacobs, K.; Wingfield, M.J. *Leptographium Species: Tree Pathogens, Insect Associates, and Agents of Blue-Stain*; American Phytopathological Society (APS Press): St. Paul, MN, USA, 2001.
54. Chieppa, J.; Chappelka, A.H.; Eckhardt, L.G. Effects of tropospheric ozone on loblolly pine seedling inoculated with root infecting ophiostomatoid fungi. *Environ. Pollut.* **2015**, *207*, 130–137. [CrossRef] [PubMed]
55. Sinclair, J.B.; Dhingra, O.D. *Basic Plant Pathology Methods*; CRC Press: Boca Raton, FL, USA, 1995.
56. Ruehle, J.L.; Marx, D.H.; Muse, H.D. Calculated nondestructive indices of growth response for young pine seedlings. *For. Sci.* **1984**, *30*, 469–474.
57. Sasek, T.W.; Richardson, C.J.; Fendick, E.A.; Bevington, S.R.; Kress, L.W. Carryover effects of acid rain and ozone on the physiology of multiple flushes of Loblolly pine seedlings. *For. Sci.* **1991**, *37*, 1078–1098.
58. Kaufmann, M.R. Evaluation of the pressure chamber technique for estimating plant water potential of forest tree species. *For. Sci.* **1968**, *14*, 369–374.
59. Box, G.E. Non-normality and tests on variances. *Biometrika* **1953**, *40*, 318–335. [CrossRef]
60. Markowski, C.A.; Markowski, E.P. Conditions for the effectiveness of a preliminary test of variance. *Am. Stat.* **1990**, *44*, 322–326. [CrossRef]
61. Samuelson, L.J.; Pell, C.J.; Stokes, T.A.; Bartkowiak, S.M.; Akers, M.K.; Kane, M.; Markewitz, D.; McGuire, M.A.; Teskey, R.O. Two-year throughfall and fertilization effects on leaf physiology and growth of loblolly pine in the Georgia piedmont. *For. Ecol. Manag.* **2014**, *330*, 29–37. [CrossRef]
62. Groninger, J.W.; Seiler, J.R.; Zedaker, S.M.; Berrang, P.C. Photosynthetic response of Loblolly pine and Sweetgum seedling stands to elevated carbon dioxide, water stress, and nitrogen level. *Can. J. For. Res.* **1996**, *26*, 95–102. [CrossRef]
63. Seiler, J.R.; Johnson, J.D. Physiological and morphological responses of three half-sib families of Loblolly pine to water-stress conditioning. *For. Sci.* **1988**, *34*, 487–495.
64. Meier, S.; Grand, L.F.; Schoeneberger, M.M.; Reinert, R.A.; Bruck, R.I. Growth, ectomycorrhizae and nonstructural carbohydrates of Loblolly pine seedlings exposed to ozone and soil water deficit. *Environ. Pollut.* **1990**, *64*, 11–27. [CrossRef]
65. Tschaplinski, T.J.; Norby, R.J.; Wullschleger, S.D. Responses of Loblolly Pine Seedlings to Elevated CO_2 and Fluctuating Water Supply. *Tree Physiol.* **1993**, *13*, 283–296. [CrossRef] [PubMed]
66. Goheen, D.J.; Cobb, F.W., Jr.; McKibbin, G.N. Influence of soil moisture on infection of Ponderosa pine by *Verticicladiella wagenerii*. *Phytopathology* **1978**, *68*, 913–916. [CrossRef]
67. Croisé, L.; Lieutier, F.; Cochard, H.; Dreyer, E. Effects of drought stress and high density stem inoculations with *Leptographium wingfieldii* on hydraulic properties of young Scots pine trees. *Tree Physiol.* **2001**, *21*, 427–436. [CrossRef] [PubMed]