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Effects of tropospheric ozone on loblolly pine seedlings inoculated with root infecting ophiostomatoid fungi

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ABSTRACT

Seedlings from four loblolly pine (*Pinus taeda* L.) families were exposed in open-top chambers to charcoal-filtered air (CF), non-filtered air (NF) or air amended with ozone to 2 times ambient (2×). Two of the families used were selected for their tolerance to fungi associated with Southern Pine Decline while two were selected for their susceptibility. Seedlings were treated with five inoculation treatments: no wound (NW), wound only (W), wound + media (WM), *Grosmannia huntii* (GH) and *Leptographium terebrantis* (LT). After 118 days of exposure (AOT40 = 31 ppm-hr^{-1} for 2× ozone) seedling volume, dry matter, chlorophyll content, water potential and lesions were measured and analyzed using ANOVA procedures. Our results indicate that seedlings selected for their susceptibility to root infecting ophiostomatoid fungi were also more sensitive to ozone. Overall lesion length was greater on seedlings exposed to elevated ozone concentrations but was not specific to either root infecting ophiostomatoid fungi.

1. Introduction

Changes in annual mean temperatures, shifts in precipitation and an increase in frequency, extremity, and intensity of storms are predicted under future climate scenarios (Paoletti et al., 2009). There is evidence that warmer temperatures have already shifted the habitats and ranges of some forest species (Kirilenko and Sedjo, 2007; Bentz et al., 2010). While direct effects of climate change on individual plants and vegetation communities may occur in the absence of plant pathogens, alterations in climate will also affect their interactions with pathogenic organisms (Garret et al., 2006).

For example, obligate biotroph infections by fungi appear to be reduced by ozone exposure and ozone-injured host tissue, while necrotrophic pathogens seem to be favored by host plants exposed to elevated ozone (Manning, 1975; Manning and von Tiedemann, 1995; Sandermann, 2000). While a consensus has yet to emerge on ozone and plant pathogen interactions (Heagle, 1973; Garrett et al., 2006), 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 and/or indirect effects.

Loblolly pine (*Pinus taeda* L.) is planted in 80% of all southern pine plantations in the Southeastern United States. and is susceptible to various biotic agents. In Alabama, several major pests include southern pine beetle (*Dendroctonus frontalis*), Ips engraver beetles (*Ips* species) and black turpentine beetle (*Dendroctonus terebrans*). Regarding plant pathogens, loblolly pine is susceptible to pitch canker fungus (*Fusarium circinatum*) and fusiform rust (*Cronartium fusiforme*). One insect and fungal association has resulted in SPD (*Leptographium* spp. and *Hylastes* spp.) (Barnard and Dixon, 1983; Price, 2008; Cordell, 1989).

Southern Pine Decline is the term for decline of, in general, southern *Pinus* species and is associated with the premature mortality of loblolly pine (Harrington and Cobb, 1983; Otrosina et al., 1997; Eckhardt et al., 2004a) and is the consequence of a series of biotic and abiotic factors. These include root pathogenic fungi (*Leptographium* and *Grosmannia* spp.), their root-feeding beetle vectors (*Hylastes salebrosus* Eichoff, *H. tenuis* Eichoff, *Hylobius pales* Herbst, and *Pachylobius picivorus* Germar), resource stress (nutrient

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deficiencies, other edaphic factors), management strategies such as overstocking, mechanical injury and fire stress (Eckhardt et al., 2010). When loblolly pine is inoculated with *Leptographium terebrantis*, the fungus causes lesions in the phloem and resin-soaking in the xylem of seedlings and mature trees of several conifers (Wingfield, 1983; Eckhardt et al., 2004b; Eckhardt et al., 2010). *Grosmannia huntii* is a related pathogen reported as being more virulent on young pine seedlings than *L. terebrantis* (Eckhardt et al., 2010).

Tropospheric ozone is produced by photochemical reactions involving hydrocarbons and nitrogen oxides and has increased at a rate of 0.3%–2.0% per year due to an increase in fossil fuel combustion (Blasing, 2009; IPCC 2013, Thompson, 1992; Vingarzan, 2004). The ubiquitous nature of this pollutant and the fact that tree response is altered by many factors (light, nutrition, moisture etc.), it is difficult to determine if the effects of ambient ozone concentrations significantly affect tree growth and productivity in the field (Chappelka and Samuelson, 1998). The effect of ozone on plant growth begins with cellular injury resulting in metabolic changes and alterations in growth if the dose is sufficient and plant repair mechanisms are overcome (Lefohn, 1992).

Plant response to pathogens has been shown to be altered by the exposure of ozone (Heagle, 1973). Ozone can change tree vigor and reduce defensive compounds which can predispose plants to infection and colonization by a pathogen (Sandermann et al., 1998). Working with loblolly pine, Carey and Kelley (1994) reported that ozone predisposed trees to the pitch canker fungus, *F. circinatum*. Cankers caused by this fungus were smaller for resistant loblolly pine families compared with susceptible loblolly pine families. Elevated ozone concentrations resulted in larger cankers caused by the pathogen regardless of tree family sensitivity to the pathogen.

There are only a few studies on ozone interactions with tree root pathogens (James et al., 1980; Lackner and Alexander, 1983; Fenn et al., 1990). Early research was conducted in Southern California with ponderosa (*Pinus ponderosa* Lawson) and Jeffrey pine (*Pinus jeffreyi* Balf.) and their relationship with the root-rot fungus *Heterobasidion irregular* Garbelotto and Otrosina, formerly *Heterobasidion annosum* (Fr) Bref. (James et al., 1980). Fenn et al. (1990) investigated the effects of ozone exposure on black stain root disease; caused by *Leptographium wageneri var. ponderosum* Harrington and Cobb of ponderosa pine. In California they reported increases in foliar injury and decreases in stem growth for inoculated seedlings. Lesion length increased with increasing ozone concentrations. Their findings indicate an interaction among these stress agents in the trees' growth. Lackner and Alexander (1983) excavated roots from air pollution sensitive and tolerant trees in the Blue Ridge Parkway in Virginia. They recovered several ophiostomatoid fungi and *Heterobasidion irregulare* from the roots of sensitive trees, but no fungi were recovered from tolerant ones.

Jones et al. (2001), in an assessment of the effect of potential future climate change scenarios for the Southeastern U.S., reported multiple factors such as ozone and changes in water availability are important. Water availability and elevated ozone concentrations can have the potential to alter loblolly pine vigor and in unison with biotic organisms, such as *L. terebrantis* or *G. huntii*, may have the potential to exacerbate pine decline and reduce productivity. The overall objective of this study was to elucidate the interactions of *L. terebrantis* and *G. huntii* in the presence of predicted climatic conditions expected in the next 50–100 years in the Southeastern U.S. Specific hypotheses include: (1) loblolly pine seedlings will be more susceptible to *L. terebrantis* and *G. huntii* when exposed to elevated ozone concentrations; (2) loblolly pine seedlings susceptible to *L. terebrantis* and *G. huntii* will be also be sensitive to ozone injury; (3) hyphal growth of *L. terebrantis* and *G. huntii* are not affected by the presence of elevated ozone.

2. Materials and methods

2.1. Study site and open-top chambers

The research site is located approximately 5 km north of the Auburn University Campus, Auburn, AL, U.S. The site contains 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 (1119 W motors), chamber plastics and Teflon tubing (Gilliland et al., 2012). Before the initiation of the study (March 2013), vegetation from each OTC was sprayed with glyphosate and removed. The bare soil was covered with landscape fabric.

2.2. Seedlings

Seedlings from four loblolly pine families were used in this study (lifted from the nursery November 2012) with two families considered tolerant to root infecting ophiostomatoid fungi (T1 and T2), while the other two were more susceptible (S1 and S2) (Singh et al., 2014). In January 2013, 2700 seedlings were planted in trade gallon pots with ProMix BX® peat-based potting mix (Premier Tech, Quebec, Canada). Seedlings were kept in a shade house and watered until mid-April when they were deployed into OTCs for acclimation before inoculations in late May 2013.

2.3. Ozone treatments

Three ozone fumigation treatments were used (replicated 3 times): (1) CF = charcoal filtered (~0.5× ambient air), representative of more pristine environments, (2) NF = non-filtered air, representative of ambient air in the Auburn, AL, U.S. area and other rural areas in the Piedmont region of the U.S., and (3) 2 × = (twice NF) representative of concentrations currently found around large urban areas such as either Atlanta, GA or Birmingham, AL (Chameides and Cowling, 1995). The 2 × is indicative of potential future ozone scenarios for rural Piedmont regions over the next 50 years (Thompson, 1992; Vingarzan, 2004).

Ozone was generated by passing pure oxygen (O_2) through a high-intensity electrical discharge source (Griffin Inc., Lodi, NJ) and added to the OTCs through Teflon tubing connected to the fan box for 12 h day^{-1} (09:00–21:00) for 7 days $week^{-1}$. Fans were turned off from 23:00–05:00 to allow natural dew formation. Ozone concentrations were monitored using U.S. EPA approved Model 49 TECO Ozone analyzers (Thermo Environmental Instruments, Inc., Hopkinton, MA). Instruments were calibrated based on U.S. EPA quality assurance guidelines. Exposures began on April 19th and ended August 14th 2013. Of the 118 days of exposure, the first 41 days were utilized to acclimate seedlings to chamber conditions and ozone concentrations. Once inoculated, seedling exposure continued for 77 more days (41 + 77 = 118 days).

Forthese data, mean 12-h (0900–21 h) ozone concentrations and peak (1-hr maximum and monthly average) ozone concentrations were calculated. Also, two cumulative exposure-response metrics were developed using the collected data: 1) AOT40 is the accumulated amount of ozone over the threshold value of 40 ppb and 2) W126, a sigmodal weighting function developed by Lefohn and Runeckles (1987). In addition, climatic data (average temperatures and rainfall) for the site were provided by Alabama Weather Information System (AWIS) Inc, Auburn, AL.

2.4. Inoculations

Stem inoculations were conducted as described by Nevill et al. (1995) from May 26–29th 2013 using the wound + inoculum

method. Five inoculation treatments were used: no wound (NW), wound only (W), wound + media (WM), *L. terebrantis* (LT) and *G. huntii* (GH). A sterile razor blade was used to cut a 5 cm vertical lesion into the bark 5 cm above the soil line to inoculate trees. Agar plugs of 2% MEA (3 mm) were placed into the wound. Media was either sterile or had *L. terebrantis* or *G. huntii* growing. All wound and inoculations were wrapped in cotton soaked with deionized water and each wound area stem region was wrapped in Parafilm® to retard desiccation.

2.5. Measurements and harvest

Seedling root collar diameter (RCD) and shoot lengths were recorded January and August of 2013 for all seedlings. Seedling volume change ($Volume_{Final} - Volume_{Initial} = Volume_{Change}$) was used to determine overall growth for individual seedlings. The equation for volume $Volume = diameter^2\ x\ height$ has been used to reliably estimate volume in seedlings and mature trees (Ruehle et al., 1984).

During seedling potting in January 2013, 40 seedlings from each family were destructively sampled and separated into needles (NE), shoot (SH), coarse roots (CR) and fine roots (FR < 2.0 mm dia). Components were placed in drying-ovens for 72 h at 70 °C and average dry matter recorded. At the end of the inoculation × exposure period, two seedlings from each treatment combination were selected for final biomass determination. Initial family means for each component (needles, coarse roots etc.) were subtracted to estimate biomass growth over the experiment, referred to as dry matter yield.

Eleven seedlings from each treatment were nondestructively sampled for relative leaf chlorophyll using a SPAD-502 chlorophyll meter (Spectrum Tech. Inc., Plainfield, IL) during the final harvest (August 2013). Needles from the first 2013 flush were selected due to their physiological maturity (Sasek et al., 1991). These same seedlings were also evaluated for incidence (% of seedlings exhibiting symptoms) and severity of visible ozone injury using a modified Horsfall-Barratt rating scale (Horsfall and Barratt, 1945; Chappelka et al., 2003). Whole plants were rated for severity of visible injury using the following categories: 0%, 1–6%, 7–25%, 26–50%, 51–75% and 76–100%. Needles displaying visible symptoms were then rated using the same scale. Visible injury was defined as chlorotic mottling, or flecking, on the needles.

The same 11 seedlings per treatment were taken to the laboratory and measured for lesion characteristics. 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). Lesion length, width and depth were measured along the stem and two pieces of stem tissue from each lesion were collected and plated on malt extract agar with cyclohexamide and streptomycin sulfate for re-isolation (Singh et al., 2014).

The remaining two seedlings from each treatment combination treatment were examined for water potential using a Scholander pressure bomb (PMS Instrument Company, Albany, OR) during final harvest. Five cm of a lateral branch was cut off each seedling and sampled as described by Kaufmann (1968). Midday sampling occurred between 1300 and 1500 h. Predawn sampling occurred between 0200 and 0500 h, however due to significant variation between replicates the data were not analyzed and thus not included in the analysis.

2.6. Data analysis

For the inoculation × family × ozone concentration study, the design was a split-split-split plot with replicates at all levels. The three concentrations of ozone, four loblolly pine families and five inoculation treatments produced sixty treatment combinations. Each treatment combination was replicated fifteen times in each chamber at the beginning of the study. Statistical analyses were conducted using SAS (SAS Institute, Inc. Cary, NC) ANOVA procedures (Glimmex procedures). Data were checked for normality using both the Kolmogorove-Smirnov and Lilliefors tests. Post-hoc Tukey (Honest Significant Difference – HSD) procedures were conducted to further investigate treatment effects. Alpha was set at 0.05. Graphics were produced using STATISTICA (StatSoft, Inc. Tulsa, OK).

3. Results

3.1. Climatic data and ozone exposures

Mean 12-h (0900–2100 h) ozone concentrations (Table 1) over the five month experiment were 14, 23 and 37 ppb for CF, NF and 2 × respectively. The seasonal 12hr AOT40 values (ppm hr^{-1}) for CF, NF and 2 × were 0.027, 1.631 and 31.277 respectively. Seasonal W126 values (ppm hr^{-1}) were 0.033, 0.423 and 21.913 for CF, NF and 2 × , respectively).

Monthly air temperatures (24-hr avg) were similar to the 30-year averages throughout the experimental period; 22.5° and 22.9 °C, respectively for April–August 2013 and the 30-yr average (Gilliland et al., 2012). Total precipitation in the Auburn, AL area for April–August 2013 was 70.1 cm was 1.3 times greater than the 30-yr average of 54.9 cm. The experimental period rainfall total was slightly greater than the 30-yr average at 14.0 cm and 11.0 cm respectively.

CF = charcoal-filtered, NF = non-filtered, 2 × – twice non-filtered. Avg. 1-h Daily Max (2×) = the average of all daily ozone peaks/maximum values in the month in 2 × ozone treatments. 1-h Monthly Max (2×) = the maximum ozone value for the entire month in the 2 × ozone treatments. AOT40 (ppb hr^{-1}) = accumulated ozone values over a threshold of 40 ppb. W126 (ppm hr^{-1}) = cumulative weighting index (Lefohn, 1992). The month of April began on the 19th and August data ended on

Table 1
12-h ozone concentration for each ozone treatment, 12-h W126 and 12-h AOT40.

Month	12-h Ozone conc. (ppb)					12-h W126 (ppm hr^{-1})			12-h AOT40 (ppm hr^{-1})		
	CF	NF	2×	Avg. 1-h daily max (2×)	1-h monthly max (2×)	CF	NF	2×	CF	NF	2×
April	17	26	41	90	97	0.000	0.000	0.010	0.000	0.038	0.666
May	19	29	48	99	154	0.000	0.001	0.019	0.027	1.205	13.116
June	15	23	39	88	140	0.023	0.310	13.980	0.000	0.337	9.378
July	12	19	30	73	140	0.010	0.112	7.909	0.000	0.051	5.606
August	9	16	28	79	102	0.000	0.000	0.004	0.000	0.000	2.511
Average	14	23	37	86	127	0.007	0.085	4.384	0.005	0.326	6.255
Sum	72	113	186	429	633	0.003	0.423	21.922	0.027	1.631	31.277

the 14th.

3.2. Seedling volume change

Overall seedling growth increased ($P < 0.0001$) when exposed to elevated ozone concentrations compared to CF and NF seedlings (Table 2, 11.7% and 8.5% respectively) across all treatments. Inoculation had no effect on seedling growth ($P = 0.585$). Regarding family differences, T1 was found to have the greatest volume growth while S2 had the least (41.4% less than T1). Families S1 and T2 grew 18.8% and 20.9% less than T1. There was a significant ozone concentration × family effect (Table 2). S1 grew more ($P < 0.011$) when exposed to elevated ozone compared to NF and CF as shown in Fig. 1 (13.7% and 8.0% respectively). S2 volume growth was not significantly different between ozone treatments ($P > 0.064$). T1 volume growth was greater ($P < 0.0001$) in 2 × chambers than those T1 seedlings grown in NF and CF (10.8% and 13.6% respectively). T2 grew 8.0% ($P = 0.024$) more in 2 × compared to T2 seedlings grown in CF but not significantly more than T2 seedlings in NF chambers ($P = 0.947$). There were no other significant interactions between treatments and treatment combinations (Table 2).

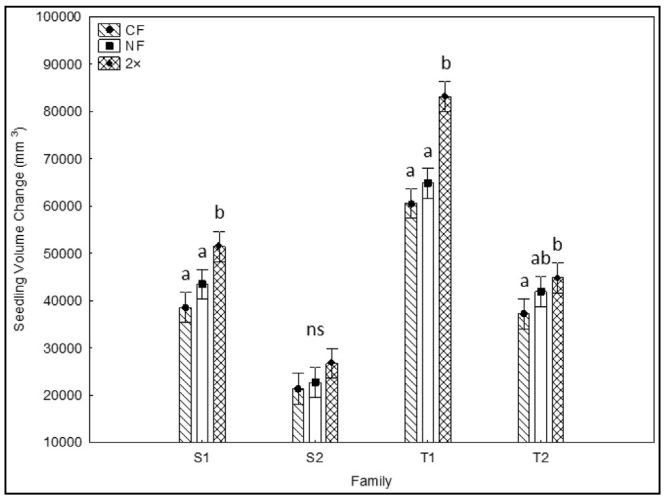

Fig. 1. Seedling volume change from January to August 2013 by family and ozone treatment. Letters are from Tukey pair-wise comparisons (specific to each family). ns = no significant difference for each family between ozone treatments. CF = charcoal filtered, NF = non-filtered, 2 × = twice NF. S1 and S2 denote families chosen for susceptibility to root infecting ophiostomatoid fungi while T1 and T2 denote families chosen for their tolerance to the fungi. Bars denote 95% confidence intervals.

4. Dry matter yield

Needle dry matter yield (DMY) and shoot DMY were found to be insignificantly different among treatments so the results for aboveground DMY (needles + shoots) are reported. Coarse root DMY and fine root DMY were found to be insignificantly different so reported are the results for belowground DMY (coarse roots + fine roots).

Seedlings exposed to elevated ozone had greater (12.8%) aboveground DMY compared to CF seedlings (Table 2). Inoculation treatments had no effect on seedling aboveground DMY ($P = 0.499$). Regarding families, T1 had the greatest aboveground DMY and S2 had the least (36.8% less than T1, $P < 0.0001$). S1 and T2 had intermediate aboveground DMY but were not different (14.6% and 19.8% less than T1 respectively, $P = 0.383$). There were no other significant interactions between treatments or treatment combinations (Table 2).

Seedlings grown in CF chambers had less belowground DMY (14.9%) compared to trees grown in 2 × chambers (Table 2). Inoculation had no effect on seedling belowground DMY ($P = 0.531$). Examining the family main effects (Table 2), T1 had the greatest belowground DMY and S2 had the least ($P < 0.0001$, 51.3% less than T1). S1 and T2 had belowground DMY between S2 and T1 but were not different ($P = 0.995$, 29.8% and 27.1% less than T1 respectively). There were no other significant interactions between treatments or treatment combinations (Table 2).

Seedlings exposed to elevated ozone had an increase in total DMY compared to seedlings grown in CF chambers (11.9%, $P = 0.003$). Inoculation had no effect on total seedling DMY ($P = 0.360$). T1 had the greatest total DMY and S2 had the least (40.7% less than T1, $P < 0.0001$). S1 and T2 had intermediate total DMY but were not different (17.7% and 21.3% less than T1

Table 2
ANOVA *P*-values for each treatment combination by measurements.

Measurement		Seedling volume change	Dry matter yield				
			Needles	Shoots	Coarse roots	Fine roots	Total
ANOVA F-Test *P*-values by treatment combination	Family	<0.0001***	<0.001**	<0.001**	<0.001**	<0.001**	<0.0001***
	O_3	<0.0001***	<0.001**	0.046*	0.682	0.913	0.008*
	Inoculation	0.585	0.523	0.499	0.531	0.884	0.360
	Family* O_3	<0.001**	0.669	0.370	0.272	0.458	0.446
	Family* Inoculation	0.041*	0.568	0.022*	0.189	0.864	0.265
	O_3* Inoculation	0.379	0.054	0.712	0.203	0.577	0.159
	Family* O_3* Inoculation	0.958	0.594	0.235	0.696	0.139	0.321

Measurement		Plant ozone injury	Leaf ozone injury	SPAD (needle greenness)	Midday water potential	Lesion length	Lesion length/seedling height
ANOVA F-Test *P*-values by treatment combination	Family	<0.0001***	<0.0001***	<0.0001***	0.001*	0.026*	<0.001**
	O_3	<0.0001***	<0.0001***	0.037*	<0.0001***	0.014*	0.002*
	Inoculation	0.151	0.091	0.942	<0.001**	<0.001**	<0.001**
	Family* O_3	<0.001**	<0.001**	0.248	0.958	0.880	0.517
	Family* Inoculation	0.597	0.268	0.182	0.421	0.211	0.324
	O_3* Inoculation	0.849	0.108	0.565	0.038*	0.304	0.183
	Family* O_3* Inoculation	0.528	0.779	0.079	0.700	0.260	0.088

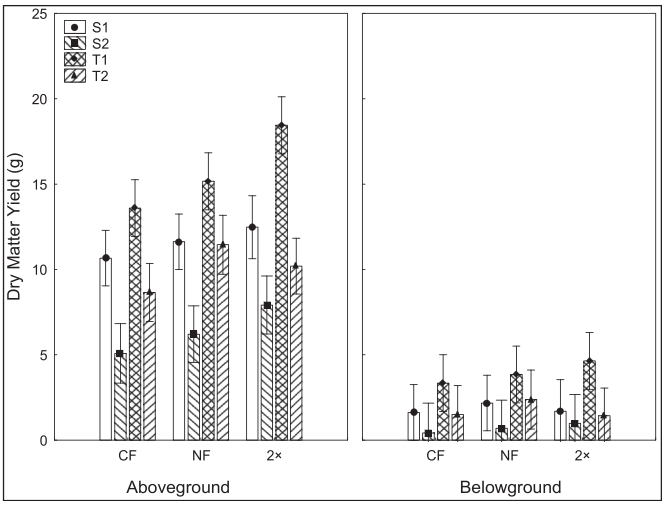

Fig. 2. Aboveground and belowground dry matter yield of seedlings by ozone treatment by family. CF = charcoal filtered, NF = non-filtered, 2 × = twice NF. Bars denote 95% confidence intervals.

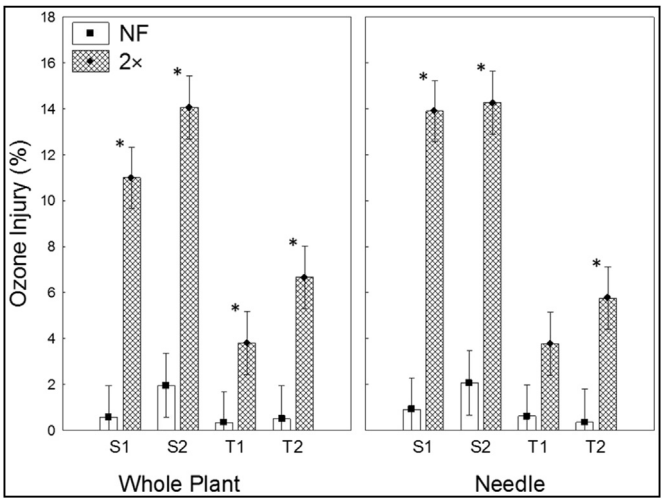

Fig. 3. Percent of seedling injured by family by ozone treatment from August 2013. A = ambient, 2 × = twice NF. S1 and S2 denote families chosen for susceptibility to root infecting ophiostomatoid fungi. T1 and T2 denote families chosen for tolerance. Asterisks denote a significant difference within each family. Bars indicate 95% confidence intervals.

respectively, $P = 0.600$, Fig. 2). There were no other significant interactions between treatments or treatment combinations (Table 2).

4.1. Visible injury

Seedlings grown in CF chambers had no visible ozone symptoms and are not included in this section. Incidence of ozone injury in families S1 and S2 were greater (52.9%) than those found in T1 and T2 (Table 3). The severity of ozone injury regarding whole plants and needles were similar (Fig. 3). Seedlings exposed to elevated ozone exhibited 10.6 × more symptoms on whole plants ($P < 0.0001$, Fig. 3). Ambient (NF) ozone exposures resulted in no significant difference in visible ozone injury between the four families tested ($P > 0.808$), however susceptible families (S1 and S2) exposed to elevated ozone (2×) had 2.4 × more injury than tolerant families ($P < 0.014$). Seedlings exposed to elevated ozone were found to have 9.9 × more ozone injury on needles ($P < 0.0001$, Fig. 3). Susceptible families (to root infecting ophiostomatoid fungi) of loblolly pine were found to have 3 × more needle ozone injury compared to tolerant families ($P < 0.0001$). Injury levels on family T1, when exposed to elevated ozone, were not different than injury levels on S2 seedlings in NF chambers ($P = 0.481$). Inoculation had no role in whole plant or leaf level ozone injury ($P > 0.091$). No other interactions were found to be significant (Table 2).

CF = charcoal filtered, A = ambient, 2 × = twice NF. S1 and S2 denote families chosen for susceptibility to root infecting ophiostomatoid fungi. T1 and T2 denote families selected for tolerance to root infecting ophiostomatoid fungi.

Table 3
Incidence (%) of seedlings with ozone injury by family at final harvest (August 2013).

Incidence (%) of seedlings with ozone injury and total samples (n)				
Pine family	CF	NF	2×	Total
S1	0% (115)	11% (118)	70% (124)	28% (357)
S2	0% (113)	27% (113)	78% (116)	35% (342)
T1	0% (115)	5% (120)	37% (115)	14% (350)
T2	0% (112)	6% (106)	42% (118)	17% (336)
Total	0% (455)	12% (457)	57% (473)	23% (1385)

4.2. Needle greenness (SPAD-502 chlorophyll meter)

Seedlings grown in CF chambers were found to have no significant difference in needle greenness compared to other chamber treatments ($P > 0.224$), however 2 × seedlings had lower needle greenness (13.7%) than those grown in NF chambers ($P = 0.021$, Fig. 4). Loblolly pine family S2 was found to have the lowest needle greenness compared to other families (5.0–8.6%, $P < 0.0001$), however, other families had no difference between them ($P > 0.194$). No other treatments or treatment combinations had significant effects on needle greenness (Table 2).

4.3. Midday water potential

Seedlings grown in NF and 2 × chambers were not different for midday water potential (Table 2). Seedlings grown in CF chambers, however, were 12.1% and 9.6% more water stressed than those

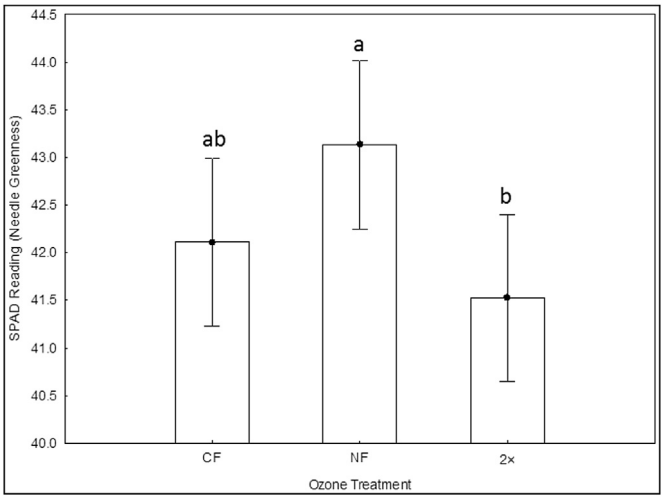

Fig. 4. Needle greenness by ozone treatment. Letters are from Tukey pair-wise comparisons. CF = charcoal filtered, NF = non-filtered, 2 × = twice NF. Bars denote 95% confidence intervals.

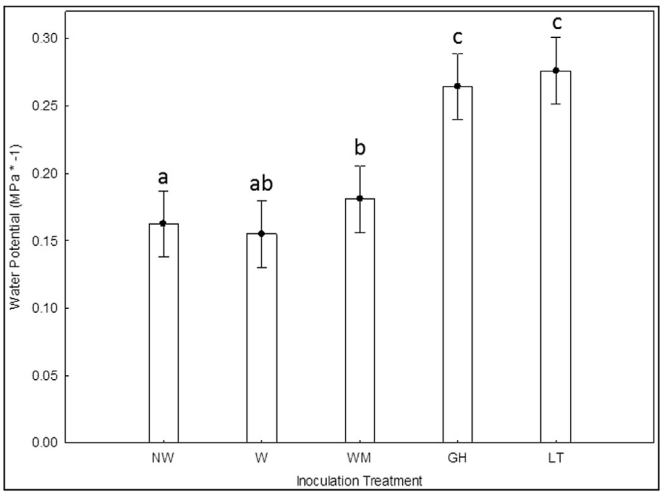

Fig. 5. Midday water potentials (megapascals*-1) of seedlings by inoculation treatment. Letters are from Tukey pair-wise comparisons. NW = no wound, W = wound only, WM = wound + media, GH = *G. huntii*, LT = *L. terebrantis*. Bars denote 95% confidence intervals.

grown in NF and 2 × treatments ($P < 0.0001$). Family T2 was found to be more water stressed than S1 and S2 seedlings (6.1% and 7.1% respectively, $P = 0.001$). Seedlings inoculated with GH and LT were more water stressed ($P < 0.0001$) than NW, W and WM controls (22.1% and 24.6% respectively, Fig. 5). Families selected for susceptibility to root infecting ophiostomatoid fungi were more water stressed when inoculated with GH or LT than the controls (S1 − 16.8% and 23.8% more stressed than the control average, $P < 0.0001$; S2 − 21.9% and 22.6% more stressed than the control average, $P < 0.002$). There were no other significant interactions between treatments and treatment combinations relevant to the studies hypotheses (Table 2).

5. Lesion measurements

Overall, lesion length and lesion length ratio were affected by ozone concentration (Table 2). Seedlings exposed to elevated ozone (2×) were found to have longer lesions ($P < 0.010$) compared to NF and CF grown seedlings; 2.4% and 2.8% smaller respectively. Lesion length ratio (lesion length/seedling height) was also greater ($P = 0.018$) in 2 × chambers compared to NF and CF grown seedlings as shown in Fig. 6; 7.5% and 7.8% smaller respectively. Seedlings grown in 2 × chambers also had greater lesion length ratios. Families S1, S2 and T1 did not significantly differ in overall lesion length ($P > 0.262$); however T2 lesion length was found to be at least 2.4% greater than all other families ($P < 0.028$). However, relative to seedling height, lesion length (lesion length ratio) was found to greatest in S2 while T1 had the smallest lesion length ratio (25.1% smaller, $P < 0.0001$). T1 was found to have the second largest lesion ratio (7.8% smaller than S2, $P = 0.044$) while S1 was found to have the third largest lesion length ratio (16.3% smaller than S2, $P < 0.0001$).

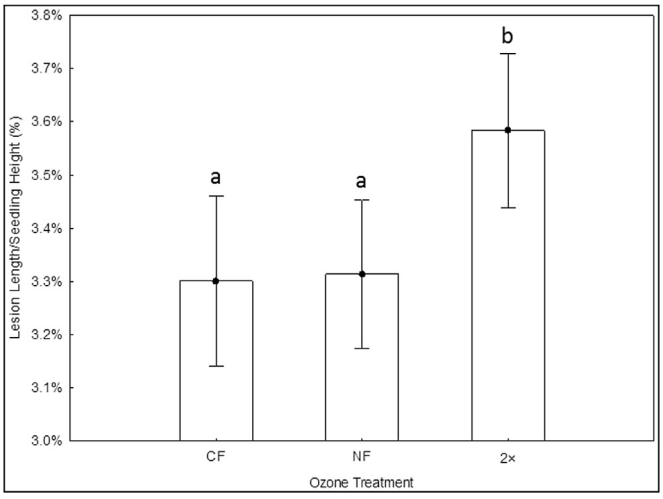

Fig. 6. Lesion lengths relative to seedling heights. Letters are from Tukey pair-wise comparisons. CF = charcoal filtered, NF = non-filtered, 2 × = twice NF. Bars denote 95% confidence intervals.

Inoculated seedling W and WM controls did not differ in lesion length ($P = 0.416$) or lesion length ratio ($P = 0.744$). Seedlings inoculated with LT were found to have lesion lengths 13.7% greater than the control average (the average of the W and WM estimated means) and 8.6% greater than GH inoculated seedlings ($P < 0.0001$). Seedlings inoculated with LT had lesion length ratios 45.7% greater than the control average and 29.0% greater than GH inoculated seedlings ($P < 0.0001$). Seedlings inoculated with GH were found to have lesion lengths 15.9% greater than W inoculated seedlings ($P = 0.005$) but were not different than WM inoculated seedlings ($P = 0.060$).

S1 seedlings inoculated with LT had lesion lengths 17.9% greater than the control average and 8.8% greater than GH inoculated seedlings ($P < 0.001$). S1 seedlings inoculated with GH were found to have lesion lengths 8.3% greater than the control average ($P < 0.046$). S1 seedlings inoculated with LT were found to have lesion length ratios 68.0% greater than the control average and 34.5% greater than GH inoculated seedlings ($P < 0.0001$), however, GH inoculated seedlings were not different than the control average ($P > 0.225$). S2 seedlings inoculated with LT had lesion lengths 11.2% greater than the control average and 9.1% greater than GH inoculated seedlings ($P < 0.001$), however GH inoculated seedling were not different than the control average ($P = 0.999$). S2 seedlings inoculated with LT were found to have lesion length ratios 29.3% greater than the control average and GH inoculated seedlings ($P < 0.008$). T1 seedlings inoculated with LT were found to have lesion lengths 15.3% greater than the control average and 10.4% greater than GH inoculated seedlings ($P < 0.001$), however, GH inoculated seedlings were not different than the control average ($P > 0.291$). T1 seedlings inoculated with LT were found to have lesion length ratios 52.1% greater than the control average and 31.7% greater than GH inoculated seedlings ($P < 0.005$). T2 seedlings inoculated with LT had lesion lengths 10.8% greater than the control average and 6.4% greater than GH inoculated seedlings ($P < 0.043$), however, GH inoculated seedlings were not different than the control average ($P > 0.799$). T2 seedlings inoculated with LT were found to have lesion length ratios 40.5% greater than the control average and 25.4% greater than GH inoculated seedlings ($P < 0.005$), however, GH inoculated seedlings were not different than the control average ($P > 0.963$). There were no other significant interactions between treatments and treatment combinations (Table 2).

6. Discussion

Although multiple exposures to ozone have resulted in reductions in growth of loblolly pine (Taylor, 1994) and other forest tree species (Leudemann et al., 2005), there is evidence that shorter durations of ozone exposure can increase aboveground growth (Spence et al., 1990). Spence et al. (1990) hypothesized that ozone injury could affect phloem loading from needles resulting in a

reduction in photosynthate transport to roots. They found a lack of transport caused accumulation of photosynthates in stems and branches which increased aboveground dry matter yield 50–60%. The loblolly pine families selected for their tolerance to root infecting ophiostomatoid fungi were larger (volume and dry matter). Perhaps the resiliency of these families is based upon reserves of carbohydrates. Carey and Kelley (1994) suggested aboveground and belowground growth competed as energy sinks. We did not observe this interaction, rather drew conclusions similar to those reported by Spence et al. (1990) where short fumigation periods caused increase aboveground growth.

The exposure to the loblolly pine families to ozone reduced loblolly pine vigor, however, lesions produced from *L. terebrantis* and *G. huntii* were no greater given increasing ozone concentrations than those from NF and CF treatments. Leudemann et al. (2009) found that ozone's ability to alter host plant susceptibility to pathogens was species-specific. The increase in lesion length was observed when all inoculation treatments were averaged indicating loblolly pine became more susceptible to mechanical stress, and possibly, root infecting ophiostomatoid fungi. The susceptible loblolly pine families (S1 and S2) exhibited greater incidence and severity of ozone injury than the tolerant families (T1 and T2). This indicates that tolerance to root infecting ophiostomatoid fungi and ozone sensitivity may be linked in the loblolly pine families tested in this experiment.

Decreased photosynthetic rates have been observed under elevated ozone conditions in loblolly pine (Taylor, 1994; Spence et al., 1990), however, short-term ozone exposure has been shown to increase photosynthetic activity through systemic-induced resistance (Sandermann et al., 1998; Pollastrini et al., 2015). Along with a decrease in chlorophyll content (needle greenness) (Richardson et al., 2002), seedlings exposed to ozone exhibited increased needle injury, typically expressed as chlorotic mottling (Grulke and Lee, 1997). The link between susceptibility to root infecting ophiostomatoid fungi seems to be linked to levels of ozone injury, indicating seedlings that are more susceptible to root infecting ophiostomatoid fungi also are more sensitive to ozone.

Ozone had no significant interactions on plant midday water potential. Rather, inoculation treatment played a significant role in water regulation. The lesions caused by the fungi *L. terebrantis* and *G. huntii* likely inhibited water uptake as all seedlings were irrigated evenly throughout the duration of the study (Wingfield, 1983; Paine, 1984; Owen et al., 1987).

Although three of the four pine families used in the study had increased growth when exposed to tropospheric ozone, we also saw larger lesion lengths relative to the seedling size. This indicates that seedlings did become more susceptible when exposed to elevated ozone concentrations. Overall lesions caused by the fungi may be inhibited in host plants exposed to ozone via systemic-induced resistance (Sandermann et al., 1998; Pollastrini et al., 2015). Carey and Kelley (1994) found similar results regarding lesion length but determined the pathogen (*F. circinatum*) caused a larger lesion when exposed to elevated ozone concentrations.

Because seedlings were observed to increase aboveground growth when exposed to elevated ozone, we felt it necessary to determine the percentage of the tree colonized (lesion length ratio). We did find that overall lesion length ratio increased with elevated ozone concentrations but the relationship was not specific to any inoculation treatment. Pollastini et al. (2015) found similar results where elevated ozone concentrations did not causes lesions from *Heterobasidion* species to be larger. Whether or not ozone affected the ability of the fungi to colonize seedling stems in our study is debatable, however, seedlings were increasingly susceptible to mechanical stress. Mechanical stress can have negative impacts on plant water transport (Sperry, 2011); however, we only saw this in seedlings inoculated with root infecting ophiostomatoid fungi.

7. Conclusions

While open-top chambers and the wound + inoculum method are useful tools in studying interactions between air quality and plant pathogens, there are many limitations (Lefohn, 1992). Outlined by Lefohn (1992), limitations include problems drawing comparisons between ozone treatments, limited space, microclimate effects on soil moisture, and pest/pathogen incidence and increased plant growth at cooler ambient temperatures. Particularly with root diseases, fungal pathogens and air quality may interact directly and cause unexplainable anomalies within the data that would not occur in field conditions (Manning and von Tiedemann, 1995).

To our knowledge, this is the first study to examine the interaction of loblolly pine seedlings inoculated with root infecting ophiostomatoid fungi and ozone. While North American emissions of ozone precursors have decreased in the last few decades, the role global emissions and transport of pollutants have become increasingly important (Cooper et al., 2012). In the Southeastern U.S., populations have increased in recent years and are expected to cause alterations to the landscape (U.S. Bureau of the Census, 2009; Wear and Greis, 2002; Milesi et al., 2003). This will likely cause increasing biogenic volatile organic compounds emissions, increased NO_x from increased automobile use and increased temperatures associated with climate change causing ozone concentrations to increase in the Southeastern U.S. (Gonzalez-Abraham et al., 2014).

In conclusion, regardless of loblolly pine family we tested, seedlings were affected on several physiological parameters by both ozone concentration and inoculation treatment. The main treatment factors affecting physiological variables were ozone concentration and pine family. There is no conclusive evidence to support that the relationship between ozone and root infecting ophiostomatoid fungi is synergistic/antagonistic. We do know that susceptibility to fungi is increased under elevated ozone growing conditions but we would recommend a longer experimental duration as well as increasing the number of families used in future studies to strengthen our conclusions regarding links between susceptibility to root infecting ophiostomatoid fungi and ozone sensitivity.

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