Environmental Pollution 207 (2015) 130–137

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

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

Jeff Chieppa [a], [*], Art Chappelka [b], Lori Eckhardt [a]

[a] Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL, USA
[b] School of Forestry and Wildlife Sciences, Auburn University, Auburn, AL, USA

ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form
28 August 2015
Accepted 30 August 2015
Available online xxx

Keywords:
Tropospheric ozone
Loblolly pine
Root infecting ophiostomatoid fungi
Leptographium terebrantis
Grosmannia huntii

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

---

[*] Corresponding author.
E-mail address: jjc0022@auburn.edu (J. Chieppa).

http://dx.doi.org/10.1016/j.envpol.2015.08.053
0269-7491/

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