# EFFECTS OF ELEVATED TROPOSPHERIC OZONE AND FLUCTUATING MOISTURE SUPPLY ON LOBLOLLY PINE SEEDLINGS INOCULATED WITH ROOT INFECTING OPHIOSTOMATOID FUNGI

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**Abstract**—Southern Pine Decline is a cause of premature mortality of *Pinus* species in the Southeastern United States. While the pathogenicity of ophiostomatoid fungi, associated with declining *Pinus* species, has been observed both in the laboratory and the field the driving mechanisms for success of fungal infection, as well as the bark-beetle vectors is less understood. The goal of this research is to provide insight into the role of future climatic conditions, specifically elevated tropospheric ozone and altered precipitation patterns, in the progression of Southern Pine Decline on loblolly pine (*Pinus taeda* L.). Two key questions were addressed: (1) will predicted concentrations of tropospheric ozone affect loblolly pine vigor and increase susceptibility to fungi associated with Southern Pine Decline?; and (2) will predicted precipitation patterns affect loblolly pine vigor and increase susceptibility to fungi associated with Southern Pine Decline? Our results indicate seedlings selected for susceptibility to root infecting ophiostomatoid fungi were more sensitive to elevated ozone than tolerant seedlings, however, neither ozone nor fluctuating moisture supply resulted in seedlings to becoming more susceptible to root infecting ophiostomatoid fungi.

## INTRODUCTION

The physical and chemical climate of the earth has changed rapidly over the last 100 years and is predicted to continue in the future (Christensen and others 2007, IPCC 2013). Global climate is changing and is apparent across a wide range of observations of which the warming over the past 50 years is primarily as a result of human activity (Walsh and others 2014.). It is likely that natural disturbances in forest ecosystems will be altered by climate change and there is evidence that warmer temperatures have already shifted suitable habitats and ranges of some forest species (Kirilenko and Sedjo 2007). Climate change-induced modifications of frequency and intensity of forest wildfires, outbreaks of insects and pathogens and extreme events such as high winds may be more important than the direct impact of higher temperatures and elevated carbon dioxide levels (Kirilenko and Sedjo 2007). The direct effects of climate change on individual plants and plant communities may occur in the absence of pathogens, but also may bring about alterations in plant metabolism that will affect their interactions with pathogens (Garrett and others 2006).

Pests that vector pathogenic fungi (e.g. ophiostomatoid fungi) (Bentz and others 2010, Kirisits 2004) are important and of concern in a changing climate. The influence of bark beetles, among other pests, is well established in the literature and typically, drought causes host plants to become stressed leading to greater infestations (Jactel and others 2012, Jones and others 2008, Klepzig and others 2004, Koricheva and others 1998). Host stress is one factor playing a part in this interaction, but insect physiology and ecologic shifts can also have drastic effects (Clarke and Fraser 2004, Gillooly and others 2001).

Water availability and tropospheric ozone are both issues of concern to the Southeastern U.S. ecosystems (Chameides and Cowling 1995, IPCC 2013, Jones and others 2001, Phillips and others 2009, Wear and Greis 2002). Water availability and ozone levels may alter loblolly pine vigor and increase susceptibility to root infecting ophiostomatoid fungi. The study addressed two scientific questions: (1) will elevated tropospheric ozone concentrations decrease loblolly pine vigor and increase susceptibility root infecting ophiostomatoid fungi? and (2) will fluctuating moisture supply decrease loblolly pine vigor and increase susceptibility to root infecting ophiostomatoid fungi?

## MATERIALS AND METHODS

The first question was addressed in 2013 utilizing large open-top chambers, three ozone concentrations and stem inoculations of four families of loblolly pine. Two of the families used were selected for tolerance

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(T1 and T2) to root infecting ophiostomatoid fungi while the others were more susceptible (S1 and S2) (Singh and others 2014). Seedlings were inoculated with *Leptographium terebrantis* Barras and Perry, *Grosmannia huntii* (Rob.-Jeffr.) Zipfel, de Beer and Wingfield and three control treatments.

Seedlings were placed in open-top chambers and exposed to three ozone treatments: CF – charcoal-filtered air (~1/2 non-filtered), NF – non-filtered air (ambient ozone), 2× - twice ambient air. Non-filtered air is representative of concentrations currently found around large urban areas such as either Atlanta, GA or Birmingham, AL (Chameides and others 1988). The 2× is indicative of potential future ozone scenarios for rural Piedmont regions over the next 50 years (Thompson 1992, Vingarzan 2004).

The second question was addressed in 2014 utilizing capped open-top chambers, three irrigation treatments and stem inoculations of four families of loblolly pine. The same families of loblolly pine were used as in the 2013 study. Seedlings were inoculated with *Leptographium terebrantis, Grosmannia huntii* and three control treatments.

Seedlings were exposed to three irrigation treatments: 3D – irrigation three days.week$^{-1}$, 4D – irrigation four days.week$^{-1}$ and 7D – irrigation seven days.week$^{-1}$. Each treatment had the same amount of moisture applied; only the intensity and frequency was altered to simulate flooding/drying periodicity (Westra and others 2014).

## RESULTS AND DISCUSSION

Although, neither elevated ozone (question 1) nor moisture stress (question 2) resulted in increased susceptibility to either *L. terebrantis* or *G. huntii*, there is evidence to suggest sensitivity to root infecting ophiostomatoid fungi is linked to abiotic stresses such as moisture stress and ozone. Tropospheric ozone induced a host response, even at low concentrations, and caused visible foliar injury. Families selected for the susceptibility to root infecting ophiostomatoid fungi had significantly greater visible ozone injury, occurring on a higher percentage of the total plants. This indicates that families of loblolly pine that are more tolerant to root infecting ophiostomatoid fungi than others may withstand short-term exposure to elevated ozone concentrations. This relationship has been seen observed in a similar study with the pathogen *Fusarium circinatum* Nirenberg and O'Donnell (Carrey and Kelley 1994).

The interaction between family susceptibility and moisture stress when challenged with the fungi is weak. Typically root pathogens and moisture stress act independently, as observed by others (Croisé and others 2001, Goheen and others 1978, Joseph and others 1998, Matusick and others 2008). Seedlings exposed to intense and infrequent irrigation events began to reduce metabolic functions towards the end of the experimental period. This strategy would likely cause seedlings to be outcompeted by other more tolerant vegetation as well as result in mortality. Family affected the response of the seedlings to water stress treatments. One of the two susceptible families had less growth with infrequent moisture events, while both tolerant families had more growth with infrequent events. Seiler and Johnson (1988) found that seed source affects the response of loblolly pine to water stress. Our results agree, however, the response is not linked to tolerance to root infecting ophiostomatoid fungi.

Tropospheric ozone and drought are considered potential threats to forests in the Southeastern U.S. (Jones and others 2001). While ozone concentrations have been moderated by air pollution legislation,[2],[3] the increasing temperatures and human population could increase ozone concentrations in the future (Gonzalez-Abraham and others 2014, Milesi and others 2003, U.S. Bureau of the Census 2009, Wear and Greis 2002). Changes in precipitation also have been observed and are expected to become more intense in the future (IPCC 2013, MacCracken and others 2000, Seager and others 2009, Wang and others 2010,). The Southeastern U.S. is already experiencing climatic changes which have had detrimental effects to both humans and natural ecosystems (Wang and others 2010).

Shifts in climate will change the way species interact with each other and individually (Manning and von Tiedemann 1995). In the Southeastern U.S., exposure to elevated concentrations of ozone over multiple seasons is predicted to decrease loblolly pine vigor and increase the tree's susceptibility to root infecting ophiostomatoid fungi. Drought and altered precipitation regimes will likely have negative impacts as well. Typically, the attributors of host-pathogen-environment interactions are easily categorized. When examining insect-fungal disease complexes, such as Southern Pine Decline, there is greater complexity to be considered.

To better understand the relationship between disease tolerance and sensitivity to moisture stress, a more thorough approach would be recommended. Using either soil moisture probes in larger planting pots (as described by Matusick and others 2008), or conducting

[2]Clean Air Act of 1970. Pub. L. 159 (July 14, 1955) 69 Stat. 322, and the amendments made by subsequent enactments. 42 U.S.C. 7401–7626. Last accessed at http://www.epw.senate.gov/envlaws/cleanair.pdf on [May 1, 2015].

[3]Clean Air Act of 1990. Pub. L. 159 (July 14, 1995) 69 Stat. 322, and the amendments made by subsequent enactments. 42 U.S.C. 7401-7626. Last accessed at http://www.epw.senate.gov/envlaws/cleanair.pdf on [May 1, 2015].

a through-fall exclusion methodology experiment could be used. Because our seedlings were potted and placed in OTCs on uneven ground, there are water runoff issues that can affect the relative humidity uniquely during different time periods of the day. The OTCs themselves can also have drying effects on warm days. This can alter the rate of evaporation from seedlings and cause a chamber effect.

Future research should focus on the effects of elevated carbon dioxide and warming temperatures with root infecting ophiostomatoid fungi. Another component missing in the climate-SPD interaction is the role of the bark beetles vectoring the ophiostomatoid fungi. Current monitoring efforts should focus on changes in the chemical and physical climate during insect monitoring trials. Elevated carbon dioxide and warming will likely alter host vigor and productivity which may increase or decrease susceptibility to biotic and abiotic agents.

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