The role of ozone in the succession of native and invasive species in Mid-Atlantic forests

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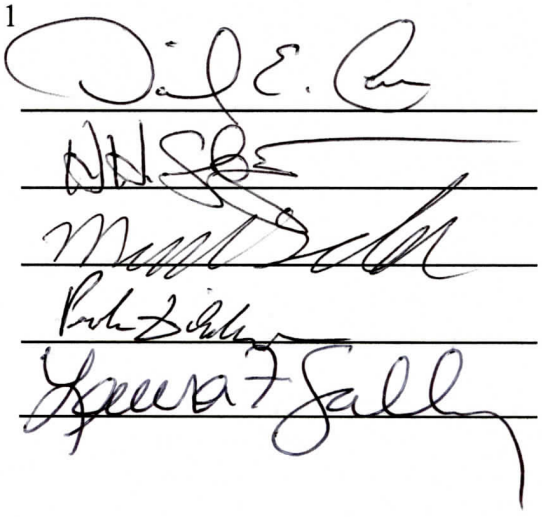

Abstract

The Mid-Atlantic region of the United States ranks as one of the top polluted regions with respect to ground-level ozone. Species-specific responses of trees to ozone may contribute to shifts in forest community structure. This study systematically compared the ozone sensitivity of native and invasive tree species common to Mid-Atlantic forests. The response of these tree species to ozone was examined in terms of gas-exchange, antioxidant capacity, and shifts in modeled forest community structure.

A common garden of 13 (8 native, 5 invasive) species was planted at Blandy Experimental Farm and exposed to three ozone treatments (20 ppb, 80 ppb, 160 ppb) during the summers of 2008-2009. Strong species-specific responses were measured in stomatal conductance, ozone uptake, and post-exposure net photosynthesis. Overall, native and invasive species did not differ in response to ozone.

To assess antioxidant protection, ascorbic acid levels within leaf extracts were quantified by hydrophilic interaction liquid chromatography. Constitutive levels of ascorbic acid differed among species. Invasive species contained higher constitutive levels of this antioxidant than native species. After ozone treatment, the majority of species had decreases in ascorbic acid. High post-exposure ascorbic acid levels were found in plants that had increases in net photosynthesis.

Species-specific ozone tolerance and life-history traits were used to parameterize an individual-based physiological tree model (TREGRO), which was linked to a spatially explicit stand gap model (ZELIG). Three forests were simulated in ZELIG, which varied in ozone level (low, medium, high). The highest abundance of native species was found

in medium treatment forests, while low and high ozone treated forests had similar presence of native and invasive species. Species composition and forest structure were affected by ozone exposure, and the response of a forest related to the ozone sensitivity and shade tolerance of modeled species.

The multispecies comparisons made throughout this study allowed for the assessment of species-specific physiological responses to ozone. These differences shifted competitive abilities, resulting in altered forest dynamics but not vast restructuring of the forest community. Future studies evaluating ozone stress on a forest community would benefit from quantifying physiological-based ozone tolerance for multiple species.

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Dedication

I dedicate this work to my mother Nancy and grandmother Johanna who inspired my passion for nature and science. They have been constant sources of encouragement, enthusiasm, and commiseration throughout the most arduous of times. I cannot thank or praise my mother enough as she has been the bulwark of my sanity, and the best mother a man could have. I also want to thank my uncle Bill for his constant support and confidence in my abilities.

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CHAPTER 1

Species-specific response to ozone among native and invasive trees

Abstract

Invasive species and air pollution currently threaten Mid-Atlantic forest systems. In this study, the effects of ozone on native and invasive species success were quantified and compared. A gas-exchange manifold was designed to test the effect of low (20 ppb), medium (80 ppb), and high (160 ppb) ozone treatments on tree leaves of a group of native and invasive species planted into a common garden at the University of Virginia's Blandy Experimental Farm. During the summers of 2008 and 2009, trees were exposed to ozone treatments for eight hours while leaf temperature, air temperature, net photosynthesis, transpiration, and ozone uptake were continuously measured. In addition to measurements taken during cuvette ozone exposures, post-exposure net photosynthesis was measured for 48 hours after ozone trials for the second year of experiments. Stomatal conductance was reduced in plants exposed to 80 ppb of ozone relative to low ozone levels of 20 ppb. In contrast, stomatal conductance in plants treated with 160 ppb ozone returned to a conductance level equivalent to the controls. Although net photosynthesis was not affected by ozone treatments during the chamber experiments, significant reductions in net photosynthesis 48 hours post-exposure were found in plants exposed to medium and high ozone levels. The reduction of stomatal conductance in leaves exposed to medium levels of ozone may have acted as a mechanism to minimize tissue damage. Acute ozone injury may have caused damage to guard cell structures in high ozone treated plants, preventing stomatal closure. Systematic differences between the responses of native and invasive species to ozone treatments did not occur, but strong species-specific trends were confirmed pointing to the potential for competitive shifts in forested areas with elevated ground-level ozone. This study points to a potential time lag

response of net photosynthesis to ozone injury, and suggests that future studies quantify the effects of ozone at least 48 hours after exposure to the pollutant.

Introduction

Two of the major problems challenging the diversity of native plant species are invasive species and air pollution (Vitousek et al. 1996, Fowler et al. 1999, Carreiro and Tripler 2005). Invasive species alter the community structure of forests by outcompeting native species, which often leads to ecosystem degradation (Vitousek et al. 1996, Mack et al. 2000). Trees in forests regularly exposed to oxidant air pollution have higher rates of mortality and shorter than average lifespan (Delucchi et al. 2002, Wittig et al. 2009). This study focuses on the effects of ozone (O_3) pollution on Mid-Atlantic deciduous forest communities and the opportunity this stress provides for invasive species.

Ozone is a phytotoxic gas that continues to increase in concentration in many areas of the world, reaching levels harmful to both plants and animals (Lovett et al. 2009). The distribution of this secondary pollutant depends upon the photochemical reactions of the primary pollutants required to form O_3, which include oxides of nitrogen (e.g., automobile exhaust, biomass burning, lightning) and volatile organic compounds (e.g., automobile exhaust, industrial processes, solvents, plants). Prior to the 18th century, background levels of O_3 were less than 10 ppb (Volz and Kley 1988), but today it is common for levels to be greater than 40 ppb in the northeast United States (Chappelka and Samuelson 1998, Skarby et al. 1998, EPA 2006, Matyssek et al. 2007). While the National Ambient Air Quality Standards (NAAQS) program has successfully reduced a

number of air pollutants, O_3 continues to rise in urban and rural areas, acting as the most important air pollutant affecting vegetation (Wittig et al. 2007).

Hourly O_3 levels above 60 ppb (AOT60), the threshold considered harmful for trees, currently affect one quarter of all forests worldwide (Fowler et al. 1999). When a tree is exposed to levels of 60 ppb, the carbon assimilation saturation level and stomatal conductance decrease by 11% and 13% on average, respectively (Wittig et al. 2007). Fowler et al. (1999) concluded that a conservative estimate of forests affected by O_3 would be double the area affected in 1990 (8.3×10^6 km^2) by the year 2100. The highest increases in concentrations of O_3 are predicted to be in the northern hemisphere (Karnosky et al. 2005). Forests are particularly at risk because air masses in forested systems have longer residence times compared to other ecosystems. The rough surface created by the forest canopy results in the greatest frictional drag of all vegetation types (Jarvis 1975, Thom and Monteith 1975). The movement of an air mass slows in proportion to the frictional drag, increasing the effects of air pollutants on trees (Fowler et al. 1999). These findings call attention to the threats facing the forests of the northeastern United States.

Among the various types of forests, hardwoods are the most susceptible to O_3-related growth reductions, possibly due to the inverse relationship between leaf longevity and O_3 sensitivity of trees (Reich 1987). Ozone affects forest trees by altering growth (Lefohn et al. 1997, Samuelson and Kelly 2001, Karnosky et al. 2005, Wittig et al. 2009), senescence (Landry and Pell 1993, Pell et al. 1997, Kitao et al. 2009), enzyme activity (Pell and Pearson 1983, Dizengremel et al. 2009, Yamaguchi et al. 2010), antioxidant levels (Pasqualini et al. 2001, Conklin and Barth 2004, Jehnes et al. 2007, Lindroth

2010), and stomatal response (Gerosa et al. 2009a, Noormets et al. 2009, Wittig et al. 2009).

Recent studies have shown varying responses to elevated O_3 levels among plant genera and species. Differing responses have been observed in antioxidant levels (Massman et al. 2000), growth rates (Musselman et al. 1994, McLaughlin and Downing 1995, Neufeld et al. 1995), leaf health (Ashmore 2005), and seedling response (Samuelson and Edwards 1993, Samuelson et al. 1996). Ozone tolerant plants tend to enhance antioxidant cycling during times of elevated O_3 exposure (Bors et al. 1989). The varying responses to O_3 among species have led for a push toward multispecies studies (Wieser and Tausz 2006, Matyssek et al. 2007).

Most research examining the effects of O_3 on trees has been conducted on a small number of species and overlooks comparisons between native and invasive species. Invasive species are those that have been introduced to an ecosystem and survive, reproduce, persist, spread and cause economic or environmental harm (Mack and Lonsdale 2001, National Invasive Species Management 2001). Many invasive species were brought to the U.S. purposefully but were never expected to escape cultivation (Ebinger 1983). Invasive species negatively affect the survival, growth, and reproduction of native species (Bratton 1982, Blossey and Notzold 1995, Vitousek et al. 1996, McDonnell et al. 1997, Mack et al. 2000, Miller et al. 2004, Hely and Roxburgh 2005, Webster et al. 2005). Broader effects of invasive species include shifts in native species diversity, forest growth and production, water and soil quality, carbon cycle, and socioeconomic value of the land (Keane and Crawley 2002, Chornesky et al. 2005).

Given species level differences in response to O_3, the impact of invasive species on our forests could change substantially as O_3 levels increase.

This study was designed to examine gas-exchange responses of common Mid-Atlantic forest species to elevated O_3 levels. Study species include early and late successional native trees as well as five invasive species. I hypothesize that invasive species will be less affected by O_3 treatments than native species due to their ability to tolerate greater levels of stress. The questions addressed in this paper are threefold: 1) what are the general physiological responses of leaves to increased O_3 exposure, 2) do species differ in their physiological responses to increased O_3 exposure and 3) do native and invasive species differ systematically in their physiological responses to increased O_3 exposure?

Methodology

Plant material

At the University of Virginia's Blandy Experimental Farm (Clarke County, Virginia, 39° 03' 55" N, 78° 03' 45" W) 117 two-year old saplings were planted during March of 2008 into a common garden. Trees were arranged into three randomized blocks aligned north to south to minimize radiation bias. Each block contained three individuals of eight native (*Acer rubrum, Carya glabra, Celtis occidentalis, Liriodendron tulipifera, Prunus serotina, Quercus alba, Quercus rubra, Robinia pseudoacacia*) and five invasive (*Acer platanoides, Ailanthus altissima, Paulownia tomentosa, Pyrus calleryana, Quercus acutissima*) species. These species include dominant-early and late successional species identified in nine urban Mid-Atlantic forests surveyed by Elton in 2006 (Dissertation

Appendix I). Species were irrigated every day during the morning and afternoon to minimize soil moisture variability throughout the growing season. See Appendix I for sources of each tree species.

Nine percent of trees required replacement after the summer of 2008 due to mortality (primarily from *Odocoileus virginianus* browsing), and these are listed in Appendix II.

Experimental design and ozone fumigation

During the summers of 2008 and 2009, leaves from all common garden saplings were treated to O_3 exposures. Each trial consisted of three leaf-cuvettes (Fuentes and Gillespie 1992) and three separate O_3 exposures (20 ppb, 80 ppb, 160 ppb). These O_3 levels were chosen to represent a range of concentrations from those currently found in forested areas to those predicted to occur in the next 50 years (Karnosky et al. 2005). A sun leaf from each of three separate trees of a given species was randomly assigned an O_3 treatment and placed inside a cuvette. Trials ran from 0800 to 1600 EDT. Two peristaltic pumps (KNF Neuberger NMP850KNDC) pulled air through a system of Teflon tubing (ID 6.35mm) connecting upstream and downstream feeds from the cuvettes to an exhaust line and a line to the gas analyzers. The flow of air through this system was regulated by a data logger (Campbell Scientific CR10) connected to two relay drivers (Campbell Scientific A21REL-12), which controlled seven 3-way solenoid valves (Asco Scientific 462312PF-12).

Ozone, carbon dioxide (CO_2) and water vapor flux rates were determined using a LICOR 6262 gas analyzer and an O_3 analyzer (2B Technologies). These instruments

sampled air from upstream and downstream lines of each cuvette for intervals of five minutes four times an hour. Six fine-wire thermocouples (5TC-TT-T Omega) measured leaf and air temperatures inside the cuvette (Fig. 1). Ozone, CO_2, water vapor partial pressures and temperature were averaged over five-minute time intervals.

In both 2008 and 2009, the response of each species to O_3 was evaluated three times for each O_3 treatment. In 2008, the first replicate (block) began on 23 June, and the last replicate ended on 4 August. In 2009, the first replicate (block) began on 12 June, and ran until 28 July. Trials did not take place on days of inclement weather (4 days in 2008, 8 days in 2009). The three replicates of each species were blocked into early, mid and late season trials. The order in which species were evaluated was randomized within each of these three blocks.

In order to calculate rates of gas-exchange, the area of the leaf contained within the cuvette was quantified. Digital photographs were taken of the leaves within each cuvette. Leaf area was calculated from these photographs using Adobe Photoshop (CS, San Jose, California). Light levels and atmospheric pressure were collected from a quantum sensor and barometer, respectively, located on a meteorological tower in a field adjacent to the experiment area. Light levels were collected to use as a potential covariate to net photosynthesis, transpiration, and O_3 flux. Atmospheric pressure was used for the calculation of physiological measurements.

To examine post-exposure effects of O_3 on net photosynthesis, an open-gas portable photosynthesis system with an infrared gas analyzer (CO750, Qubit Systems Inc., Ontario, Canada) was used one and two days after initial O_3 exposures in 2009. To measure post-exposure net photosynthesis, the same leaves that had been exposed to low,

medium, or high levels of O_3 in the fumigation experiment were selected. The Qubit leaf chamber was clamped to each leaf for five minutes and measured every other hour from 0800-1600 EDT on the first and second day after fumigation trials.

Physiological measurements

The mass balance method was used to calculate flux density (F_x) of CO_2 (g m^{-2} s^{-1}), water vapor (g m^{-2} s^{-1}) and O_3 (g m^{-2} s^{-1}) from the differences in gas concentrations (parts of gas per air ratio) that enter the cuvette (X_i) and concentrations leaving the cuvette (X_o), flow rate (*f*, m^3 s^{-1}), molecular weight (M_x, g mol^{-1}), atmospheric pressure (p, Pa), leaf area (A, m^2), the universal gas constant (R, J mol^{-1} K^{-1}) and temperature (T, K) (Amiro et al. 1984):

$$F_x = (X_i - X_o) \bullet \frac{fM_x p}{ART} \quad (1)$$

To determine the stomatal conductance of H_2O vapor (g_s, mol m^{-2} s^{-1}) of the leaf within the cuvette, the Ball & Berry equation was used (Ball et al. 1987):

$$g_s = \frac{mA_n h_s}{C_s} + g_0 \quad (2)$$

where net photosynthesis (A_n, μmol m^{-2} s^{-1}), percent relative humidity (h_s), and CO_2 concentration at the leaf surface (C_s, μmol m^{-2}) were directly measured or calculated from measurements during cuvette experiments. The slope, *m* (9.50 μmol m^{-2}) is referred to as

the stomatal sensitivity factor (Baldocchi and Meyers 1998), and the intercept, g_o (0.07 mol m^{-2} s^{-1}) is the stomatal conductance as A_n approaches zero.

Stomatal conductance was strongly correlated with the rate of evapotranspiration (r=0.81, p<0.0001). A well-coupled relationship between g_s and evapotranspiration characterizes typical leaf function.

Fluxes (F_x) and g_s were averaged for the entire trial period (eight hours), leaving a single mean for physiological calculations attributed to each plant within the common garden. Cumulative ozone uptake (COU) values were obtained by the summing of hourly mean O_3 flux measurements across the entire eight-hour trial.

Statistical analyses

To test for relationships among gas-exchange variables, Pearson's product moment correlation coefficients (r) between all measured and calculated variables were estimated with SAS PROC CORR (version 9.1.3; SAS Institute, Cary, NC, USA). To determine statistical significance, Sequential Bonferroni (Rice 1989) was used with the correlation analysis.

A repeated measures mixed-model ANOVA was used with the PROC MIXED function in the SAS statistical package to test for the effect of O_3 on gas-exchange measurements. Because each tree was measured in 2008 and again in 2009, the tree served as the subject in the repeated measures analysis and year was the time effect. The fixed effects in this model included native/invasive status, O_3 treatment and the interaction of status*O_3 treatment. The random effects included block, year, species nested within status, and the treatment*species interaction effect. This model was

applied to the following dependent variables: stomatal conductance (g_s), net photosynthesis, O_3 flux, and cumulative O_3 uptake (COU). Reduced models were evaluated in order to compute log-likelihood ratio tests (G) to evaluate the significance of the random effects (Littell et al. 2006).

A repeated measures mixed-model ANOVA was used with the PROC MIXED function in SAS to test for the effect of O_3 treatment on post-exposure gas-exchange measurements made with the Qubit photosynthesis system. Each tree was measured repeatedly every two hours from 0800-1600 EDT during the 48 hours following chamber experiments and measurements were averaged for each tree, thus each tree (nested within species) served as the subject in the repeated measures analysis. The time variable used in the analysis was day (e.g., day 1 or day 2 after chamber trials). The dependent variable utilized by the model was net photosynthesis. The fixed effects in the model included O_3 treatment, time and O_3 treatment*time interaction. Random variables included block, species and species*O_3 treatment interaction. Contrasts were run to evaluate all pertinent comparisons.

Beyond native and invasive species groups, other combinations of species were compared, but significant differences between groups were not found (data not shown). These groups included ruderal versus non-ruderal and early versus late successional species.

Results

Effect of ozone on leaf function

To establish the effect of O_3 on leaf-level gas-exchange processes, the concentrations of CO_2, O_3, and water vapor were quantified throughout cuvette experiments. During the 8 hr O_3 exposure trial, stomatal conductance (g_s) was significantly lower at medium O_3 treatments than either low or high treatments (Fig. 2a, Table 1). Net photosynthesis was not affected by O_3 treatments (Fig. 2b, Table 2). The O_3 flux rate for leaves increased with increasing O_3 concentration (Fig. 3). Cumulative ozone uptake (COU) of leaves exposed to medium and high treatments exhibited increases with increases in O_3 concentrations (Fig. 4).

The O_3 flux rate of all individuals was weakly negatively correlated with net photosynthesis (r=-0.22, p=0.0006). This pattern was stronger for plants treated with high O_3 treatments (r=-0.44, p<0.0001). The correlations between O_3 flux and net photosynthesis were not significant when analysis was restricted to low and medium treatments. Cumulative O_3 uptake and g_s were moderately negatively correlated (r=-0.32, p<0.0001).

To understand the relationship between g_s and COU across O_3 treatments, a heterogeneity of slopes model was used to test for an O_3 treatment*COU interaction. A significant O_3 treatments*COU interaction indicated that the effect of COU on g_s differed significantly among O_3 treatments ($F_{2,176}$=7.97, p<0.0001). The slope of the regression of g_s on COU for the low treatment was not significantly different from zero (Fig. 5a, slope=-0.02, t=-1.40, df=76, p=0.1632), but the slopes of medium (Fig. 5b, slope=-0.04, t=-2.98, df=76, p=0.0033) and high (Fig. 5c, slope=0.04, t=3.60, df=76, p=0.0004)

treatments were significant. The interaction was produced by the opposing direction of the slopes of medium and high treatments. A stomatal response was seen where medium treatments caused reduction in g_s with increasing COU, but high treatments caused increases in g_s with continued increases in COU.

To examine post-exposure effects of O_3, net photosynthesis rates were measured one and two days after the 8 hr O_3 trials. A time*O_3 interaction ($F_{2,113} = 5.19$, p=0.0070) indicated that net photosynthesis after ozone exposure was lower in leaves exposed to medium and high O_3 treatments compared to low O_3 exposed leaves (Fig. 6). When net photosynthesis was examined one day after exposures, low, medium and high treatments did not differ, echoing the patterns found in the chamber experiments. However, day two marked a significant decrease in net photosynthesis for plants exposed to medium treatments when compared to low O_3 treated plants ($F_{1,22}$=4.10, p<0.0436), and an even greater decrease in net photosynthesis for plants exposed to high treatments when compared to the low O_3 treated plants ($F_{1,22}$=5.44, p=0.0214). This indicates a potential time lag response of growth in leaves exposed to pollutants.

Species-specific effects and interactions

Species varied significantly in g_s (Table 1), net photosynthesis (Table 2), O_3 flux rates (Table 3), and COU (Table 1). The species with the highest g_s values were native ruderal and invasive species including *R. pseudoacacia*, *P. tomentosa*, and *A. altissima*. Those species with the lowest g_s were all native species including *P. serotina*, *Q. alba*, and *A. rubrum*. The species with the highest net photosynthesis during the cuvette trials were *P. calleryana*, *R. pseudoacacia*, and *L. tulipifera*. The species with the lowest net

photosynthesis during cuvette experiments were *Q. alba*, *A. rubrum*, and *Q. rubra*. The species with the highest O_3 flux rates were *P. serotina*, *A. altissima*, and *Q. acutissima*. *Quercus alba*, *A. platanoides*, and *P. calleryana* had the lowest O_3 flux rates. Species that had the highest COU included *A. altissima*, *C. occidentalis*, and *C. glabra*. The species that had the lowest COU included *P. calleryana*, *Q. alba*, *and L. tulipifera* (Table 2).

Net photosynthesis showed a significant species*O_3 interaction. Despite the variation in species response to O_3 treatments, a few species showed similar net photosynthesis trends along the low, medium, and high O_3 gradient (Fig. 7). The most common pattern was a decrease of net photosynthesis from low to medium treatments with an increase in net photosynthesis at high treatment. This "V" is shallow for most species, but *C. glabra* and *Q. acutissima* have very deep "V" patterns showing a large reduction in net photosynthesis in the medium treatment. *Liriodendron tulipifera* showed a strong decrease in net photosynthesis in the medium O_3 treatment, but there was no recovery at the high treatment, forming an "L" pattern. *Prunus serotina* showed a pattern of an inverted "V", where net photosynthesis increased from low to medium treatment, and decreased at high O_3 treatment. One other unique pattern is shown by *P. calleryana*, which had net photosynthesis increasing from low to medium to high O_3 treatments.

Examination of net photosynthesis 1-2 days after O_3 exposure illustrated a species*time interaction ($F_{12,113}$=5.19, p=0.0070). The species with the largest reductions in net photosynthesis at day one were *A. platanoides*, *P. tomentosa*, and *Q. alba*. Two species had overall increases in net photosynthesis during day one including *C. occidentalis* and *P. serotina*. Two days after O_3 exposures, the species with the greatest

overall reductions in net photosynthesis were *A. platanoides*, *P. tomentosa*, and *C. glabra*. The species with the smallest reductions in net photosynthesis at day two were *Q. rubra*, *A. altissima*, *and Q. acutissima*. *Acer rubrum* showed an overall increase in net photosynthesis 48 hours after O_3 exposures.

Native and invasive species

Native and invasive species did not differ systematically in their response throughout the gas-exchange trials. Net photosynthesis (Fig. 8) was slightly higher for the invasive species than native species, but this difference was not statistically significant. The g_s (Table 1), O_3 flux rate (Table 3) and COU analyses (Table 1) all failed to show significant differences between the native and invasive species. The native/invasive status*O_3 interaction was not significant for any of the dependant variables, indicating that native and invasive species were responding similarly to O_3 treatments.

Post-exposure net photosynthesis showed no significant difference between native and invasive species (Table 3).

Discussion

Effect of ozone on leaf function

A reduction of stomatal conductance (g_s) is a common response of plants to elevated O_3 levels, and Reich (1987) has suggested that this is a mechanism of O_3 tolerance. The reduction of g_s limits the exposure of mesophyll cells to oxidative pollution, but this often results in a reduction of net photosynthesis. Decreases in g_s may

also result from the impairment of potassium ion channels that control stomatal openings (Torsethaugen et al. 1999).

Ozone treatments in this study affected stomata functioning in a nonlinear fashion. The medium treatment showed significantly lower rates of g_s than the low treatment, but the high treatment did not differ significantly from the low treatment. The findings in the medium treatment support Reich's (1987) O_3 tolerance theory, where reductions in g_s limit exposure to O_3. However, even with reduced g_s limiting the exposure to O_3 for medium treated leaves, lasting damage did occur as noted 48 hours after exposure to O_3.

The O_3 tolerance theory put forth by Reich (1987) may not apply if stomata are injured. The increased g_s in the high O_3 treatment may indicate an acute injury threshold (Sharkey et al. 1996, Herbinger et al. 2005, Gregg et al. 2006, Haikio et al. 2009) that occurred somewhere between the medium (80 ppb) and high O_3 treatment (160 ppb). Acute damage to stomata can prevent closure of stomata in high stress environments. Acute injury has been noted at 150 ppb in *Fagus sylvatica* (Herbinger et al. 2005). *Populus deltoides* exposed to three-times ambient O_3 levels had reductions in growth, but increased g_s (Gregg et al. 2006). High concentrations of O_3 have also caused increases in g_s of a group of grasses (Mills et al. 2009).

The mechanism controlling increases in g_s after acute injury is currently unknown. When injury occurs, plants are unable to maintain maximum photosynthetic carbon fixation rates which may prevent the activation of antioxidant defense systems (Herbinger et al. 2005).

The overall strong linear relationship between O_3 exposure and cumulative ozone uptake (COU) (Fig. 4) would seem to argue against stomatal regulation of O_3 exposure and acute O_3 damage at high treatments. However, if plants have values of COU proportional to g_s, then we would conclude that stomata are responding similarly across treatments. If one examines stomatal behavior only within medium treatments, leaves maintain high g_s at low COU but have significant reductions in g_s at damaging levels of COU (>30 mmol m^{-2} day^{-1}, Fig. 8b, Gerosa et al. 2009b). If one considers only the high treatments, leaves also show reductions in g_s at mid-range COU but increase g_s at high COU values (>50 mmol m^{-2} day^{-1}) (Fig. 8c). The failure of stomata to close at high COU values is evidence for acute O_3 damage that disables stomata regulation.

When assessing gas-exchange response, examining COU may be more relevant than the atmospheric concentrations assigned by O_3 treatments (Karlsson et al. 2004, Wieser and Tuasz 2006, Gerosa et al. 2009b). Stomatal conductance showed a negative correlation with COU, but the correlation was lower than noted in previous studies (Novak et al. 2005, Moraes et al. 2006). The lower correlation noted in this study is likely due to the variability of response among the different species and the large range of COU values. Previous studies did not find values of COU in the range determined to cause acute O_3 injury (>50 mmol m^{-2} day^{-1}). Acute O_3 injury would cause an increase in g_s at high COU, weakening the overall correlation.

When one compares net photosynthesis across O_3 treatments, the same pattern emerges as with g_s: a drop at medium and a recovery at high O_3 treatments. But, when the treated leaves were observed 48 hours later, reductions in net photosynthesis for high O_3 treated leaves occurred. This points to damage incurred by photosynthetic cells at the

time of exposure. The initial increase in net photosynthesis at high treatments was a likely product of unregulated g_s, which led to increased exposures of O_3 for high treated leaves, producing greater long-term damage. After 48 hours, photosynthetically active cells were likely degraded and unable to contribute to the process of photosynthesis.

This study makes a strong case for the role of time after exposure to O_3 as a contributor to variation in gas-exchange processes. A large number of experiments have measured gas-exchange responses simultaneously with O_3 treatments (Fuentes and Gillespe 1992, Schaub et al. 2005, Bagard et al. 2008), while other studies have measured growth response after O_3 exposure (Hanson et al. 1994, Herbinger et al. 2005, Moraes et al. 2006). The results for this study show clear differences in conclusions between gas-exchange results taken during the O_3 exposure trials and those taken after the exposures. Therefore, plant gas-exchange data tested against O_3 uptake values from the same time period could be misleading, as plants exhibited a delayed response to O_3 through 48 hours. Though studies have shown decreases in photosynthesis during O_3 exposures, most investigators manipulated concentrations of O_3 in the range of the medium treatment used in the present study (Bortier et al. 2000, Wittig et al. 2007, Haikio et al. 2009). This study noted increased g_s in acute O_3 injured plants, which allowed for increased access to carbon resources. The ability of a plant to mitigate damage associated with high O_3 levels likely results in the noted time lag for the high O_3 treated plants. This study suggests that researchers should implement a post-exposure timeline of gas-exchange measurements at least 48 hours after O_3 treatment. The study of visible leaf injury also supports such recommendations, as leaves commonly display visible injury three to six days after O_3 exposure (Esposito et al. 2009).

Species-specific effects and interactions

Trait differences among species are key in determining competitive interactions and successional dynamics of a forest community. The variation in gas-exchange among species would logically lead to species-specific responses to air pollutants (Reich 1987). The species utilized for this study varied significantly in basic gas-exchange parameters such as g_s, net photosynthesis, and O_3 flux rates, which were anticipated due to species-specific responses noted in the literature (Kozlowski et al. 1991, Bassow and Bazzaz 1997, Lambers et al. 1998, Novak et al. 2005, Orendovici-Best et al. 2008, Bassin et al. 2009, Luedemann et al. 2009). Moreover, species showed a strong interaction with O_3 treatments on the effect of both net photosynthesis and g_s. If all species reacted similarly to O_3 treatments, then we could assume that O_3 does not affect the succession and persistence of species in a forest. However, noting the variation found among species response to O_3 in this study, it is critical to detail the ability of each species to cope with elevated O_3 treatments in order to predict potential changes in forest dynamics.

Five of the species in this study showed significant negative correlations between g_s and COU: *A. altissima*, *A. platanoides*, *C. occidentalis*, *R. pseudoacacia,* and *L. tulipifera*. This indicates that these species regulate their stomata in response to intracellular O_3 levels. Two species (*A. altissima*, *C. occidentalis*) exhibited reductions in net photosynthesis as O_3 flux increased, presumably due to the reduction in g_s The remaining species (*A. platanoides*, *R. pseudoacacia*, *L. tulipifera*) did not suffer reductions in net photosynthesis with increasing O_3 flux, suggesting that they would perform best in areas that regularly experience 40-80 ppb concentration of O_3. The

remaining species showed no or weakly positive correlations between g_s and O_3 flux, indicating that these species were not regulating their stomata in response to O_3 flux.

The high O_3 treatment mimicked extreme O_3 events anticipated to increase in the near future. The species that had the largest reductions of net photosynthesis in response to O_3 flux were *A. altissima*, *P. tomentosa*, and *C. occidentalis*. Stomatal regulation failed within the high treatment when COU reached 50 mmol m^{-2} day^{-1}. Under these circumstances, O_3 tolerance might best be evaluated by examining post-exposure net photosynthesis. Only three species showed significant positive correlations of O_3 flux and post-exposure net photosynthesis: *C. glabra*, *P. calleryana*, and *Q. alba*. Overall, species were negatively affected by the high O_3 treatments illustrating the potential for shifts in forest dynamics.

Native and invasive species

Several studies have attributed the success of invasive species to higher photosynthetic rates (Baruch and Goldstein 1999, Durand and Goldstein 2001, McDowell et al. 2002). In the present study, native and invasive species did not differ in net photosynthesis. The large number of species included here reduces sampling bias and creates a more robust model to evaluate the reliability of growth rate for measuring invasive species success. One criticism of this study may be that comparisons are not phylogenetically controlled, but even when congeneric pairs (*Quercus* spp. and *Acer* spp.) are isolated and tested statistically, there is no difference in net photosynthesis between native and invasive species. However, a number of studies (Schierenbeck and Marshall 1993, Mack 1996, McDowell et al. 2002) that have phylogenetically controlled

native and invasive comparisons have found that invasive species do have higher photosynthetic rates than native counterparts.

All other physiological traits and effects of O_3 were similar in native and invasive species. Other ecological groupings of species (early vs. late successional, ruderal vs. non-ruderal) also failed to produce meaningful patterns (data not presented). In a forest system, the species in this study may vary in their response to O_3 due to other factors (e.g., soil, light, herbivory, and water conditions) that affect plant growth, which were controlled in this study. For example, herbivory in combination with elevated O_3 may reduce the growth of native species to an extent that allows invasive species to flourish.

Conclusions

Native and invasive species of Mid-Atlantic forests did not differ significantly in gas-exchange measurements or in their response to O_3 treatments. Some of the native species (*P. serotina*, *R. pseudoacacia*) may have confounded the comparison of native and invasive species, however, when ruderal versus non-ruderal species groups were compared no significant differences were found.

The species-specific responses noted in this study point to potential shifts in plant performance in the presence of elevated O_3. Variation in the response of species to O_3 predicts changes in forest dynamics. Scaling up these physiological responses to O_3 could give insight on the restructuring role O_3 may play in forests.

Many studies have shown the importance of using cumulative ozone uptake as a measure of the influence of O_3 on plants. Importantly, this study showed acute injury was delayed after O_3 exposure for a wide variety of trees common to the Eastern United

States. This research will help to influence policy on O_3 sensitivity indices for plant species categorization. The variation among the response of the 13 species suggests that competition will occur as O_3 levels continue to rise worldwide. In order to assess the long-term impacts and potential competitive shifts in response to O_3, future studies could employ forest models that extrapolate species-specific O_3 response data to a broad time and spatial scale.

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Literature Cited

Amiro, B D, T J Gillespie, and G W Thurtell. 1984. Injury response of *Phaseolus vulgaris* to ozone flux density. *Atmospheric Environment* 18: 1207-1215.

Ashmore, M R. 2005. Assessing the future global impacts of ozone on vegetation. *Plant Cell and Environment* 28: 949.

Bagard, M, D L Thiec, E Delacote, M P Hasenfratz-Sauder, J Banvoy, J Gérard, P Dizengremel, and Y Jolivet. 2008. Ozone-Induced changes in photosynthesis and photorespiration of hybrid poplar in relation to the developmental stage of the leaves. *Physiologia Plantarum* 134: 559-74.

Baldocchi, D, and T Meyers. 1998. On using eco-physiological, micrometerological and biogeochemical theory to evaluate carbon dioxide, water vapor and trace gas fluxes over vegetation: a perspective. *Agricultural and Forest Meteorology* 90:1-25.

Ball, J T, I E Woodrow, and J A Berry. 1987. A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. *Progress in Photosynthesis Research* 4:221-224.

Baruch, Z and G Goldstein. 1999. Leaf construction cost, nutrient concentration, and net CO_2 assimilation of native and invasive species in Hawaii. *Oecologia* 121:183-192.

Bassin, S, R A Werner, K Sörgel, M Volk, N Buchmann, and J Fuhrer. 2009. Effects of combined ozone and nitrogen deposition on the in situ properties of eleven key plant species of a subalpine pasture. *Oecologia* 158: 747-56.

Bassow, S L and F A Bazzaz. 1997. Intra- and inter-specific variation in canopy photosynthesis in a mixed deciduous forest. *Oecologia* 109: 507-515.

Blossey, B and R Notzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: A hypothesis. *The Journal of Ecology* 83: 887-889.

Bors, W, C Langebartles, C Michel, and H J R Sandermann. 1989. Polyamines as radical scavengers and protectants against ozone damage. *Phytochemistry* 28: 1589-1595.

Bortier, K, R Ceulemans, and L de Temmerman. 2000. Effects of ozone exposure on growth and photosynthesis of beech seedlings (*Fagus sylvatica*). *New Phytologist* 146: 271-280.

Bratton, S P. 1982. The effects of exotic plant and animal species on nature preserves. *Natural Areas Journal* 2: 3-13.

Carreiro, M M and C E Tripler. 2005. Forest remnants along urban-rural gradients: Examining their potential for global change research. *Ecosystems* 8: 568-582.

Chappelka, A H and L S Samuelson. 1998. Ambient ozone effects on forest trees of the eastern United States: a review. *New Phytologist* 139: 91-108.

Chornesky, E A, A M Bartuska, G H Aplet, K O Britton, J Cummings-Carlson, F W Davis, J Eskow, D R Gordon, K W Gottschalk, R A Haack, R N Mack, F J Rahel, M A Shannon, L A Wainger, and T B Wigley. 2005. Science priorities for reducing the threat of invasive species to sustainable forestry. *Bioscience* 55: 335-348.

Conklin, P L and C Barth. 2004. Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. *Plant, Cell and Environment* 27: 959-970.

Delucchi, M A, J J Murphy, and D R McCubbin. 2002. The health and visibility cost of air pollution: A comparison of estimation methods. *Journal of Environmental Management* 64: 139-52.

Dizengremel, P, D Le Thiec, M P Hasenfratz-Sauder, M N Vaultier, M Bagard, and Y Jolivet. 2009. Metabolic-Dependent changes in plant cell redox power after ozone exposure. *Plant Biology* 11:35-42.

Durand, L Z and G Goldstein. 2001. Photosynthesis, photoinhibition, and nitrogen use efficiency in native and invasive tree ferns in Hawaii. *Oecologia* 126: 345-354.

Ebinger, J E. 1983. Exotic shrubs: A potential problem in natural area management in Illinois. *Natural Areas Journal* 3: 3-6.

Esposito, M P, M L Ferreira, S M R Sant'Anna, M Domingos, and S R Souza. 2009. Relationship between leaf antioxidants and ozone injury in *Nicotiana tabacum* 'bel-w3' under environmental conditions in Sao Paulo, SE - Brazil. *Atmospheric Environment* 43: 619-623.

Fowler, D, J N Cape, M Coyle, C Flechard, J Kuylenstierna, K Hicks, D Derwent, C Johnson, and D Stevenson. 1999. The global exposure of forests to air pollutants. *Water, Air, and Soil Pollution* 116: 5-32.

Fuentes, J D and T J Gillespie. 1992. A gas exchange system to study the effects of leaf surface wetness on the deposition of ozone. *Atmospheric Environment. Part A, General Topics* 26: 1165-1173.

Gerosa, G, R Marzuoli, R Desotgiu, F Bussotti, and A Ballarin-Denti. 2009a. Validationof the stomatal flux approach for the assessment of ozone visible injury in young forest trees. Results from the TOP (transboundary ozone pollution) experiment at Curno, Italy. *Environmental Pollution* 157: 1497-505.

Gerosa, G, A Finco, S Mereu, M Vitale, F Manes, and A Ballarin-Denti. 2009b. Comparison of seasonal variations of ozone exposure and fluxes in a mediterranean holm oak forest between the exceptionally dry 2003 and the following year. *Environmental Pollution* 157: 1737-44.

Gregg, J W, C G Jones, and T E Dawson. 2006. Physiological and developmental effects of O_3 on cottonwood growth in urban and rural sites. *Ecological Applications* 16: 2368-81.

Haikio, E, M Makkonen, R Julkunen-Tiitto, J Sitte, V Freiwald, T Silfver, V Pandey, E Beuker, T Holopainen, and E Oksanen. 2009. Performance and secondary chemistry of two hybrid aspen (*Populus tremula* L. X *Populus tremuloides* michx.) Clones in long-term elevated ozone exposure. *Journal of Chemical Ecology* 35: 664-678.

Hanson, P J, L J Samuelson, S D Wullschleger, T A Tabberer, and G S Edwards. 1994. Seasonal patterns of light-saturated photosynthesis and leaf conductance for mature and seedling *Quercus rubra* L. Foliage: Differential sensitivity to ozone exposure. *Tree Physiology* 14: 1351-66.

Harley, P C and J D Tenhunen. 1991. Modeling the photosynthetic response of C3 leaves to environmental factors. *Modeling Crop Photosynthesis—From Biochemistry to Canopy.*17-39.

Hely, S E L and S H Roxburgh. 2005. The interactive effects of elevated CO_2, temperature and initial size on growth and competition between a native C3 and an invasive C3 grass. *Plant Ecology* 177: 85-98.

Herbinger, K, Ch Then, M Löw, K Haberer, M Alexous, N Koch, and K Remele. 2005. Tree age dependence and within-canopy variation of leaf gas exchange and antioxidative defence in *Fagus sylvatica* under experimental free-air ozone exposure. *Environmental Pollution* 137: 476-82.

Jarvis, P G. 1975. Water transfer in plants. In: D.A. de Vries and N. H. Afgan, Editors, *Heat and Mass Transfer in the Biosphere. I. Transfer Processes in Plant Environment,* Halsted, Washington , DC, 369-394.

Jehnes, S, G Betz, G Bahnweg, K Haberer, H Sandermann, and H Rennenberg. 2007. Tree internal signalling and defence reactions under ozone exposure in sun and shade leaves of european beech (*Fagus sylvatica* L.) Trees. *Plant Biology* 9: 253-264.

Karlsson, P E, J Uddling, S Braun, M Broadmeadow, S Elvira, B S Gimeno, and D Le Thiec. 2004. New critical levels for ozone effects on young trees based on AOT40 and simulated cumulative leaf uptake of ozone. *Atmospheric Environment* 38: 2283-2294.

Karnosky, D F, K S Pregitzer, D R Zak, M E Kubiske, G R Hendrey, D Weinstein, M Nosal, and K E Percy. 2005. Scaling ozone responses of forest trees to the ecosystem level in a changing climate. *Plant, Cell and Environment* 28: 965.

Keane, R M and M J Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17: 164-170.

Kitao, M, M Löw, C Heerdt, T E E Grams, K H Häberle, and R Matyssek. 2009. Effects of chronic elevated ozone exposure on gas exchange responses of adult beech trees (*Fagus sylvatica*) as related to the within-canopy light gradient. *Environmental Pollution* 157: 537-44.

Kozlowski, T T, P J Kramer, and S G Pallardy. 1991. The physiological ecology of woody plants. *Tree Physiology* 8: 213.

Lambers, H, F S Chapin, and T L Pons. 1998. *Plant Physiological Ecology.* Springer.

Landry, L G and E J Pell. 1993. Modification of Rubisco and Altered Proteolytic Activity in O_3-Stressed Hybrid Popla*r (Populus Maximowizii* x *Trichocarpa) Plant Physiology* 101: 1355-1362.

Lefohn, A S, W Jackson, D S Shadwick, and H P Knudsen. 1997. Effect of surface ozone exposures on vegetation grown in the southern Appalachian Mountains: Identification of possible areas of concern. *Atmospheric Environment* 31: 1695-1708.

Lindroth, R L. 2010. Impacts of elevated atmospheric CO_2 and O_3 on forests: Phytochemistry, trophic interactions, and ecosystem dynamics. *Journal of Chemical Ecology* 36: 2-21.

Littell, R C. 2006. *SAS for Mixed Models.* SAS Publishing.

Loehle, C. 2003. Competitive displacement of trees in response to environmental change or introduction of exotics. *Environmental Management* 32: 106-115.

Lovett, G M, T H Tear, D C Evers, S E G Findlay, B J Cosby, J K Dunscomb C T Driscoll and K C Weathers. 2009. Effects of air pollution on ecosystems and biological diversity in the eastern United States. *Annals of the New York Academy of Science* 1162: 99-135.

Löw, M, K-H Häberle, C R Warren, and R Matyssek. 2007. O_3 flux-related responsiveness of photosynthesis, respiration, and stomatal conductance of adult *Fagus sylvatica* to experimentally enhanced free-air O_3 exposure. *Plant Biology* 9: 197-206.

Löw, M, K Herbinger, A J Nunn, K H Häberle, M Leuchner, C Heerdt, H Werner, P Wipfler, H Pretzsch, M Tausz, and R Matyssek. 2006. Extraordinary drought of 2003 overrules ozone impact on adult beech trees (*Fagus sylvatica*). *Trees-Structure and Function* 20: 539-548.

Luedemann, G, R Matyssek, J B Winkler, and T E E Grams. 2009. Contrasting ozone x pathogen interaction as mediated through competition between juvenile European beech (*Fagus sylvatica*) and norway spruce (*Picea abies*). *Plant and Soil* 323:47-60.

Mack, R N and W M Lonsdale. 2001. Humans as global plant dispersers: Getting more than we bargained for. *Bioscience* 51: 95-102.

Mack, R N, D Simberloff, W M Lonsdale, H Evans, M Clout, and F A Bazzaz. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689-710.

Mack, R N. 1996. Predicting the identity and fate of plant invaders: Emergent and emerging approaches. *Biological Conservation* 78: 107-121.

Manning, W J. 2005. Establishing a cause and effect relationship for ambient ozone exposure and tree growth in the forest: Progress and an experimental approach. *Environmental Pollution* 137: 443-54.

Massman, W J, R C Musselman, and A S Lefohn. 2000. A conceptual ozone dose-response model to develop a standard to protect vegetation. *Atmospheric Environment* 34: 745-759.

Matyssek, R, A Bytnerowicz, P-E Karlsson, E Paoletti, M Sanz, M Schaub, and G Wieser. 2007. Promoting the O_3 flux concept for European forest trees. *Environmental Pollution* 146: 587-607.

Matyssek, R, M S Günthardt-Goerg, T Keller, and C Scheidegger. 1991. Impairment of gas exchange and structure in birch leaves (*Betula pendula*) caused by low ozone concentrations. *Trees-Structure and Function* 5: 5-13.

Mills, G, F Hayes, S Wilkinson, and W J Davies. 2009. Chronic exposure to increasing background ozone impairs stomatal functioning in grassland species. *Global Change Biology* 15: 1522-1533.

McDonnell, M J, S T A Pickett, P Groffman, P Bohlen, R V Pouyat, W C Zipperer, R W Parmelee, M M Carreiro, and K Medley. 1997. Ecosystem processes along an urban-to-rural gradient. *Urban Ecosystems* 1: 21-36.

McDowell, S CL. 2002. Photosynthetic characteristics of invasive and noninvasive species of *Rubus* (*Rosaceae*). *American Journal of Botany* 89: 1431-1438.

McLaughlin, S B and D J Downing. 1995. Interactive effects of ambient ozone and climate measured on growth of mature forest trees. *Nature* 374: 252-254.

Mehlhorn, H, M Lelandais, H G Korth, and C H Foyer. 1996. Ascorbate is the natural substrate for plant peroxidases. *Federation of European Biochemical Societies Letters* 378: 203-6.

Mehlhorn, H and A R Wellburn. 1987. Stress ethylene formation determines plant sensitivity to ozone. *Nature* 327: 417-418.

Miller, K E and D L Gorchov. 2004. The invasive shrub, *Lonicera maackii*, reduces growth and fecundity of perennial forest herbs. *Oecologia* 139: 359-75.

Moraes, R M, P Bulbovas, C M Furlan, M Domingos, S T Meirelles, W B C Delitti, and M J Sanz. 2006. Physiological responses of saplings of *Caesalpinia echinata* lam., A Brazilian tree species, under ozone fumigation. *Ecotoxicology and Environmental Safety* 63: 306-12.

Musselman, R C, T Younglove, and P M McCool. 1994. Response of *Phaseolus vulgaris* L. To differing ozone regimes having identical total exposure. *Atmospheric Environment* 28: 2727-2731.

Neufeld, H S, E H Lee, J R Renfro, W D Hacker, and B H Yu. 1995. Sensitivity of seedlings of black cherry (*Prunus serotina* ehrh.) To ozone in Great Smoky Mountains National Park. I. Exposure-Response curves for biomass. *New Phytologist* 130: 447-459.

Noormets, A, O Kull, A Sôber, M E Kubiske, and D F Karnosky. 2009. Elevated CO_2 response of photosynthesis depends on ozone concentration in aspen. *Environmental Pollution* 158: 992-999.

Novak, K, M Schaub, J Fuhrer, J M Skelly, C Hug, W Landolt, P Bleuler, and N Kräuchi. 2005. Seasonal trends in reduced leaf gas exchange and ozone-induced foliar injury in three ozone sensitive woody plant species. *Environmental Pollution* 136: 33-45.

Orendovici-Best, T, J M Skelly, D D Davis, J A Ferdinand, J E Savage, and R E Stevenson. 2008. Ozone uptake (flux) as it relates to ozone-induced foliar symptoms of *Prunus serotina* and *Populus maximowizii x trichocarpa*. *Environmental Pollution* 151: 79-92.

Pasqualini, S, P Batini, L Ederli, A Porceddu, C Piccioni, F De Marchis, and M Antonielli. 2001. Effects of short-term ozone fumigation on tobacco plants: Response of the scavenging system and expression of the glutathione reductase. *Plant, Cell and Environment* 24: 245-252.

Pell, E J, C D Schlagnhaufer, and R N Arteca. 1997. Ozone-Induced oxidative stress: Mechanisms of action and reaction. *Physiologia Plantarum* 100: 264-273.

Pell, Eva J and N S Pearson. 1983. Ozone-Induced reduction in quantity of ribulose-1,5-bisphosphate carboxylase in alfalfa foliage. *Plant Physiology* 73: 185-187.

Reich, P B. 1987. Quantifying plant response to ozone: A unifying theory. *Tree Physiology* 3: 63-91.

Rice, W R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223-225.

Samuelson, L J and J M Kelly. 2001. Scaling ozone effects from seedlings to forest trees. *New Phytologist* 149: 21-41.

Samuelson, L J, J M Kelly, P A Mays, and G S Edwards. 1996. Growth and nutrition of *Quercus rubra* L. Seedlings and mature trees after three seasons of ozone exposure. *Environmental Pollution* 91: 317-23.

Samuelson, L J and G S Edwards. 1993. A comparison of sensitivity to ozone in seedlings and trees of *Quercus rubra* L. *New Phytologist* 125: 373-379.

Schaub, M, J M Skelly, J W Zhang, J A Ferdinand, J E Savage, R E Stevenson, D D Davis, and K C Steiner. 2005. Physiological and foliar symptom response in the crowns of *Prunus serotina*, *Fraxinus americana* and *Acer rubrum* canopy trees to ambient ozone under forest conditions. *Environmental Pollution* 133: 553-67.

Schierenbeck, K A and J D Marshall. 1993. Seasonal and diurnal patterns of photosynthetic gas exchange for *Lonicera sempervirens* and *L. japonica* (*Caprifoliaceae*). *American Journal of Botany* 80: 1292-1299.

Sharkey, T D, E L Singsaas, P J Vanderveer, and C Geron. 1996. Field measurements of isoprene emission from trees in response to temperature and light. *Tree Physiology* 16: 649-54.

Skarby, L, H Ro-Poulsen, F A M Wellburn, and L J Sheppard. 1998. Impacts of ozone on forests: a European perspective. *New Phytologist* 139: 109-122.

Thom, A S and J L Monteith. 1975. Vegetation and the atmosphere. *Momentum, Mass and Heat Exchange,* 57-109.

Torsethaugen, G, E J Pell, and S M Assmann. 1999. Ozone inhibits guard cell K+ channels implicated in stomatal opening. *Proceedings of the National Academy of Sciences* 96: 13577.

Vitousek, P M, C M D'Antonio, L L Loope, and R Westbrooks. 1996. Biological invasions as global environmental change. *American Scientist* 84: 468-478.

Volz, A and D Kley. 1988. Evaluation of the Montsouris series of ozone measurements made in the nineteenth century. *Nature* 332: 240-242.

Webster, C R, M A Jenkins, and S Jose. 2006. Woody invaders and the challenges they pose to forest ecosystems in the eastern United States. *Journal of Forestry* 104: 366-374.

Webster, C R, K Nelson, and S R Wangen. 2005. Stand dynamics of an insular population of an invasive tree, *Acer platanoides*. *Forest Ecology and Management* 208: 85-99.

Wieser, G and M Tausz. 2006. *Trees at Their Upper Limit: Treelife Limitation at the Alpine Timberline.* Springer Verlag.

Wittig, V E, E A Ainsworth, S L Naidu, D F Karnosky, S P Long, and A R S USDA. 2009. Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: A quantitative meta-analysis. *Global Change Biology* 15: 396-424.

Wittig, V E, E A Ainsworth, and S P Long. 2007. To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last 3 decades of experiments. *Plant, Cell and Environment* 30: 1150-62.

Yamaguchi, M, M Watanabe, H Matsumura, Y Kohno, and T Izuta. 2010. Effects of ozone on nitrogen metabolism in the leaves of *Fagus crenata* seedlings under different soil nitrogen loads. *Trees-Structure and Function* 24: 175-184.

Table 1: Analysis of variance for the effects of block (random effect), native/invasive status (fixed effect), ozone treatment (fixed effect), and species (random effect) on the following gas-exchange processes measured during the cuvette experiments: stomatal conductance (g_s), net photosynthesis (A_n), ozone flux, and cumulative ozone uptake (COU). Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.

			g_s	A_n	O_3 flux	COU
Source	ndf	ddf	*F or* X^2	*F or* X^2	*F or* X^2	*F or* X^2
Block	2	22	---	---	---	---
Status	1	11	3.66	1.28	0	0.12
Ozone	2	22	6.06**	2.94	40.35***	31.22***
Status*Ozone	2	22	0.56	0.21	0.1939	1.18
Species(Status)	11	---	31.47***	2.88*	13.44**	6.90**
Ozone*Species(Status)	22	---	4.60*	14.29***	---	---

Table 2: Species means and standard deviations for net photosynthesis (A_n, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mmol m^{-2} s^{-1}), ozone flux (nmol m^{-2} s^{-1}), cumulative ozone uptake (COU, mmol m^{-2} s^{-1}), net photosynthesis one day after ozone trials (A_{D1}, μmol m^{-2} s^{-1}), and net photosynthesis two days after ozone trials (A_{D2}, μmol m^{-2} s^{-1}).

Species	A_n	g_s	O_3 flux	*COU*	A_{D1}	A_{D2}
A. altissima	16.35 (3.08)	175.91 (1.13)	13.91 (4.17)	26.01 (2.13)	14.79 (2.89)	14.23 (3.16)
A. platanoides	15.08 (5.23)	169.02 (1.08)	8.80 (4.97)	16.56 (2.69)	10.51 (1.84)	10.03 (2.33)
A. rubrum	10.94 (3.69)	146.94 (1.12)	11.92 (4.30)	21.72 (1.66)	9.81 (3.34)	17.53 (7.67)
C. occidentalis	13.85 (3.45)	149.90 (1.11)	12.82 (4.25)	23.81 (2.28)	16.78 (1.94)	15.36 (1.92)
C. glabra	12.64 (6.83)	160.77 (1.20)	13.14 (3.82)	23.72 (1.72)	9.43 (3.82)	8.47 (3.81)
L. tulipifera	17.34 (5.76)	154.47 (1.20)	9.30 (3.45)	15.84 (1.06)	15.14 (3.55)	13.80 (3.90)
P. tomentosa	13.04 (6.28)	177.68 (1.15)	11.97 (6.03)	18.84 (1.30)	8.47 (3.41)	8.05 (3.15)
P. serotina	13.05 (4.65)	129.02 (1.04)	14.17 (6.08)	22.56 (3.96)	15.96 (3.08)	15.14 (3.85)
P. calleryana	17.94 (3.82)	175.91 (1.11)	9.08 (4.77)	15.05 (2.43)	15.09 (2.11)	14.53 (1.52)
Q. acutissima	13.08 (6.07)	151.41 (1.09)	13.14 (6.07)	21.72 (0.38)	11.96 (4.27)	10.44 (3.48)
Q. alba	9.79 (3.65)	134.29 (1.03)	8.45 (3.97)	15.60 (2.86)	6.29 (2.19)	5.68 (2.68)
Q. rubra	11.61 (2.54)	148.41 (1.12)	11.33 (5.42)	19.45 (2.86)	10.93 (2.53)	9.60 (2.00)
R. pseudoacacia	17.69 (5.68)	194.42 (1.19)	10.44 (3.75)	19.10 (1.72)	15.10 (2.54)	14.63 (3.04)

Table 3: Analysis of variance for the effects of block (random effect), ozone treatment (fixed effect), native/invasive status (fixed effect), time after exposure (fixed effect), and species (random effect) on post-exposure net photosynthesis (A_{Dx}). Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.

Source	ndf	ddf	*F or* X^2
Block	2	---	---
Ozone	2	24	2.56
Status	1	113	0.12
Time	1	113	0.53
Time*Ozone	2	113	5.19**
Species(Status)	12	---	1.94
Ozone*Species(Status)	24	---	22.21***

Figure Legend:

Figure 1: Gas-exchange manifold including three leaf cuvettes (High, Medium, Low), seven 3-way solenoid valves, air mixing chamber, two peristaltic pumps, gas analyzers (Licor 6262, O_3 analyzer), ozone generator (O_3 Supply) and Teflon tubing connecting all elements. Arrows indicate direction of airflow.

Figure 2: Plant gas-exchange to low, medium, and high ozone treatments a) stomatal conductance (g_s), and b) net photosynthesis (A_n). Error bars represent 95% confidence intervals. Different letters on error bars indicate significant difference at $p<0.05$ tested using Tukey's post hoc comparisons.

Figure 3: The daily mean ozone flux rate for all samples by ozone treatment. Error bars represent 95% confidence intervals.

Figure 4: Cumulative ozone uptake averages for each species by ozone treatment. Error bars represent 95% confidence intervals.

Figure 5: Stomatal conductance rates for a) low, b) medium, and c) high ozone treatment leaves by cumulative ozone uptake.

Figure 6: Net photosynthesis (A_n) of trees one and two day after ozone exposures. Error bars represent 95% confidence intervals. Significant differences were found for Day 2 medium and high treated leaves when compared to low treated leaves.

Figure 7: Net photosynthesis (A_n) by species at three ozone treatment levels for *A. altissima* (AA), *A. platinoides* (AP), *A. rubrum* (AR), *C. glabra* (CG), *C. occidentalis* (CO), *L. tulipifera* (LT), *P. calleryana* (PC), *P. serotina* (PS), *P. tomentosa* (PT), *Q. acutissima* (QAC), *Q. alba* (QAL), *Q. rubra* (QR), *R. pseudoacacia* (RP).

Figure 8: Net photosynthesis (A_n) by native and invasive species at each ozone treatment. Error bars represent 95% confidence intervals.

Figure 1:

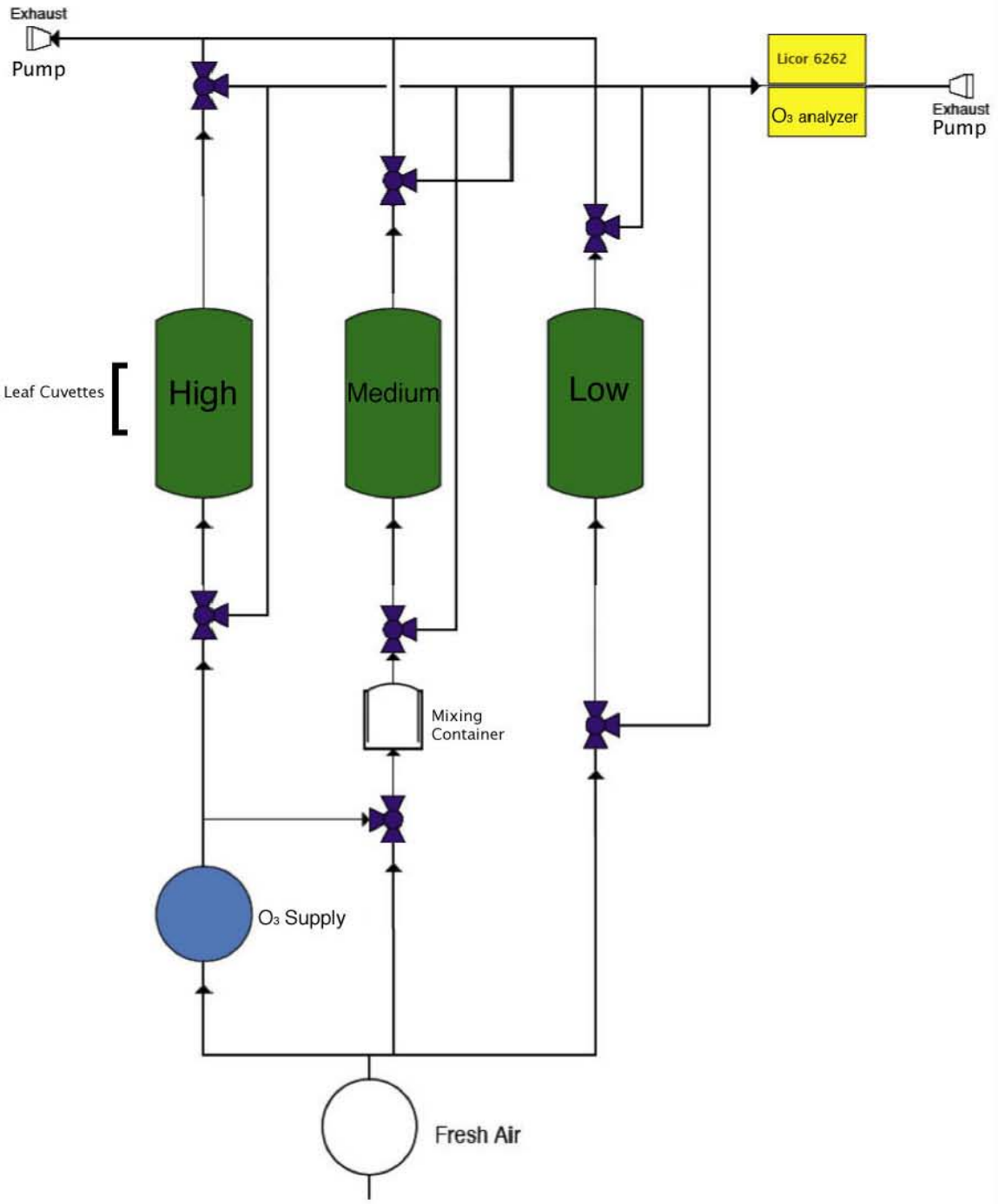
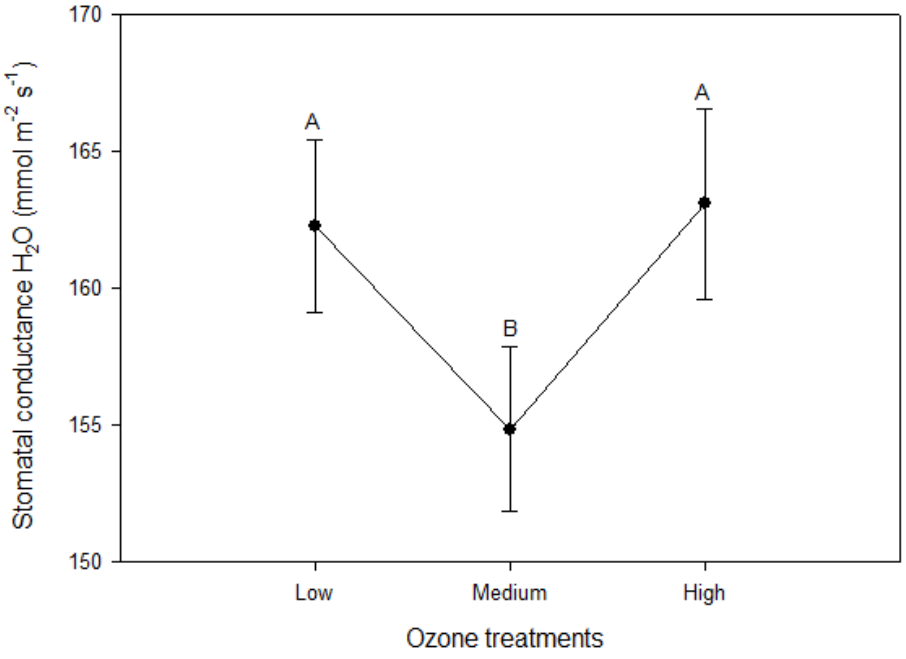

Exhaust
Pump
Licor 6262
O3 analyzer
Exhaust
Pump
Leaf Cuvettes
High
Medium
Low
Mixing
Container
O3 Supply
Fresh Air

Figure 2a:


170
165
160
155
150
Stomatal conductance H_2O (mmol m^{-2} s^{-1})
A
B
A
Low
Medium
High
Ozone treatments

b:

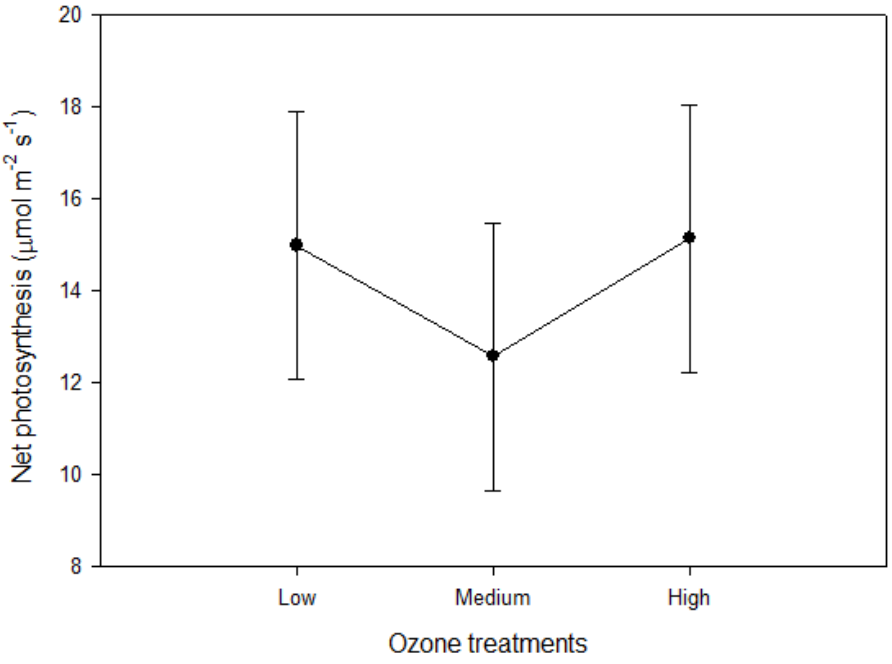

20
18
16
14
12
10
8
Net photosynthesis (μmol m^{-2} s^{-1})
Low
Medium
High
Ozone treatments

Figure 3:

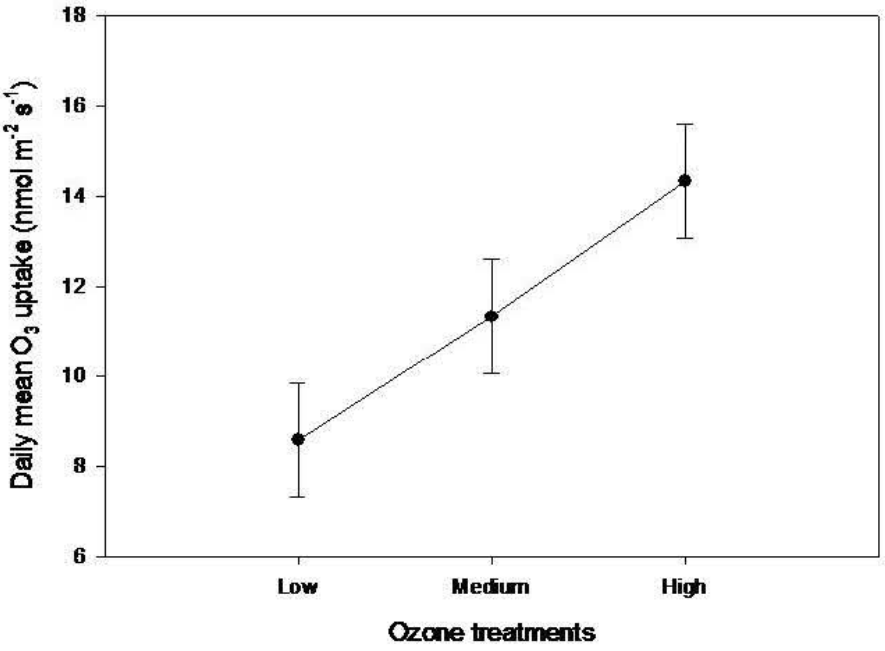

18
16
14
12
10
8
6
Daily mean O_3 uptake (nmol m^{-2} s^{-1})
Low
Medium
High
Ozone treatments

Figure 4:

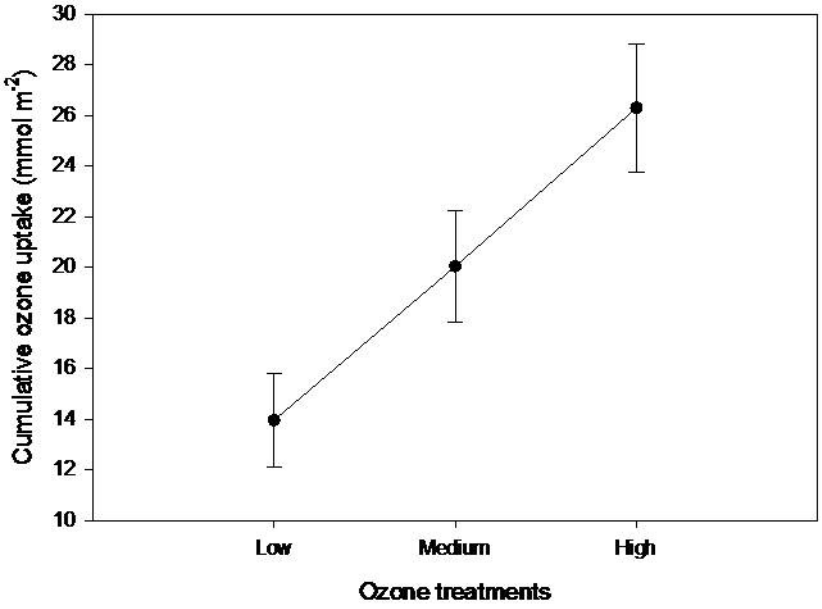
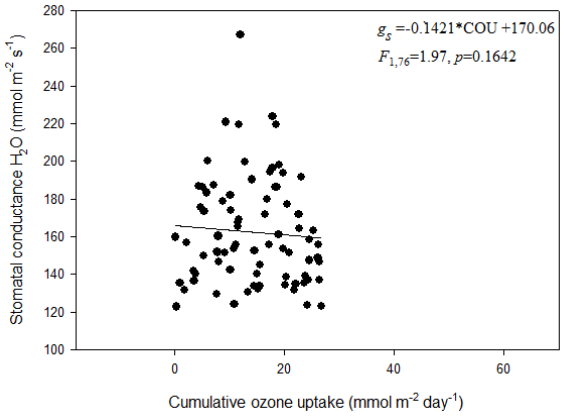
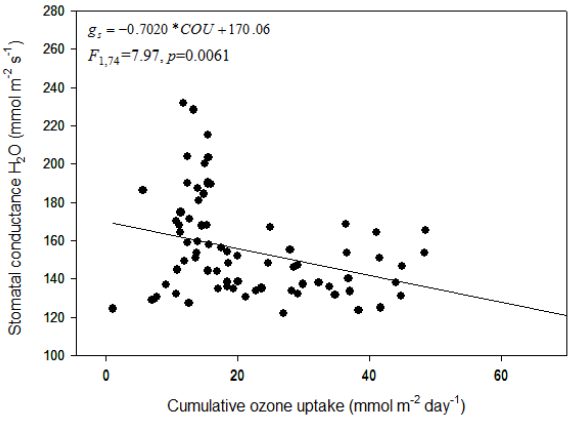

30
28
26
24
22
20
18
16
14
12
10
Cumulative ozone uptake (mmol m-2)
Low
Medium
High
Ozone treatments

Figure 5a:


g_s =-0.1421*COU +170.06
$F_{1,76}$=1.97, p=0.1642
Stomatal conductance H_2O (mmol m^{-2} s^{-1})
Cumulative ozone uptake (mmol m^{-2} day^{-1})

b:


g_s = −0.7020 *COU +170.06
$F_{1,74}$=7.97, p=0.0061
Stomatal conductance H_2O (mmol m^{-2} s^{-1})
Cumulative ozone uptake (mmol m^{-2} day^{-1})

c:

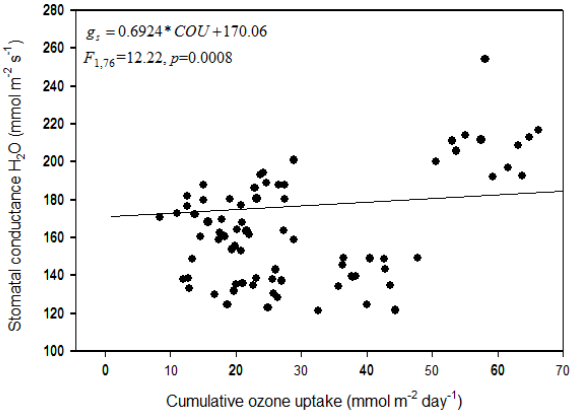

g_s = 0.6924 * COU +170.06
$F_{1,76}$=12.22, p=0.0008
Stomatal conductance H_2O (mmol m^{-2} s^{-1})
Cumulative ozone uptake (mmol m^{-2} day^{-1})

Figure 6:

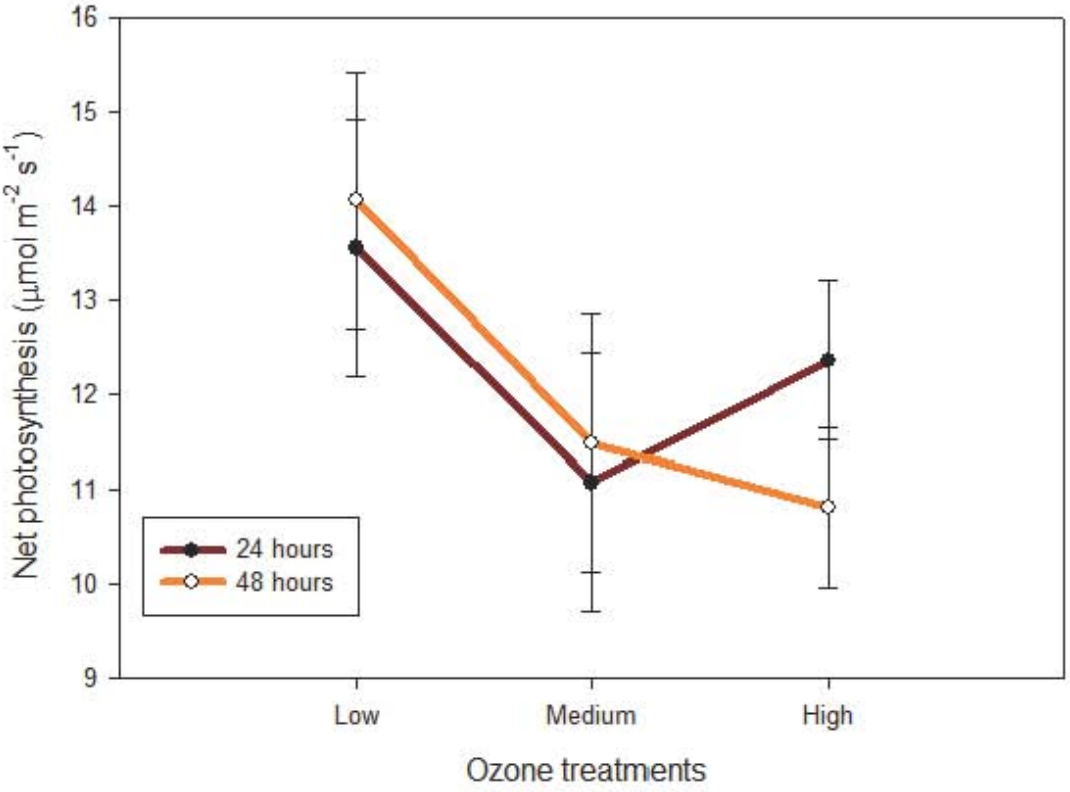

16
15
14
13
12
11
10
9
Net photosynthesis (µmol m-2 s-1)
24 hours
48 hours
Low
Medium
High
Ozone treatments

Figure 7:

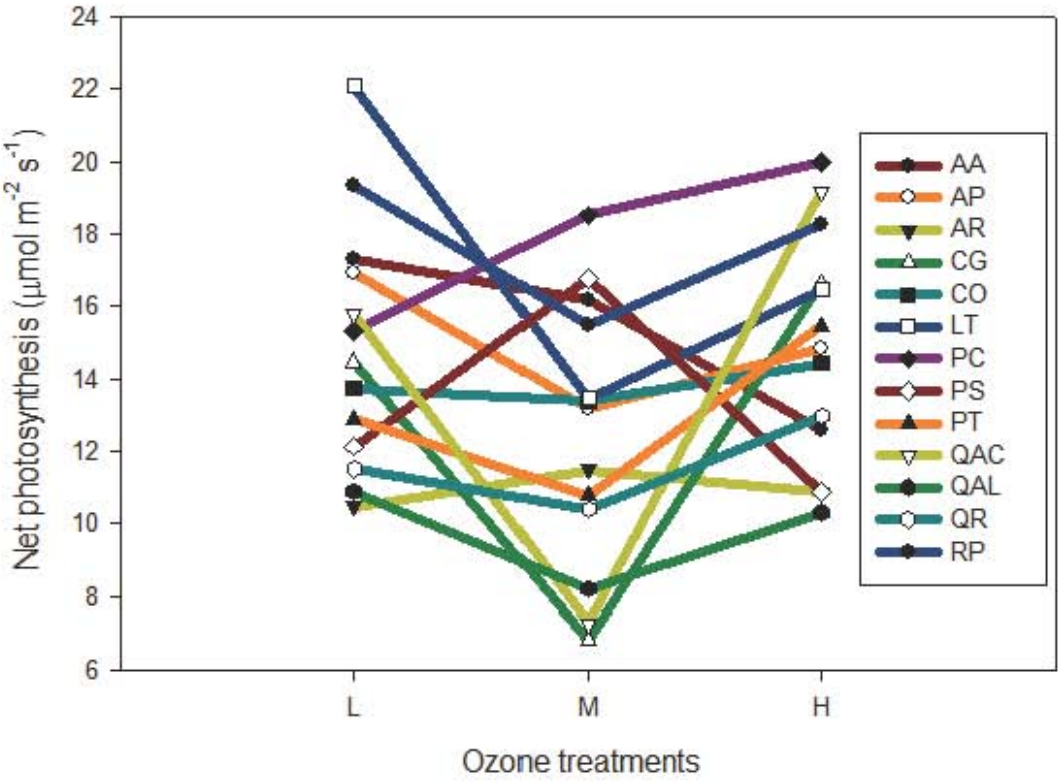
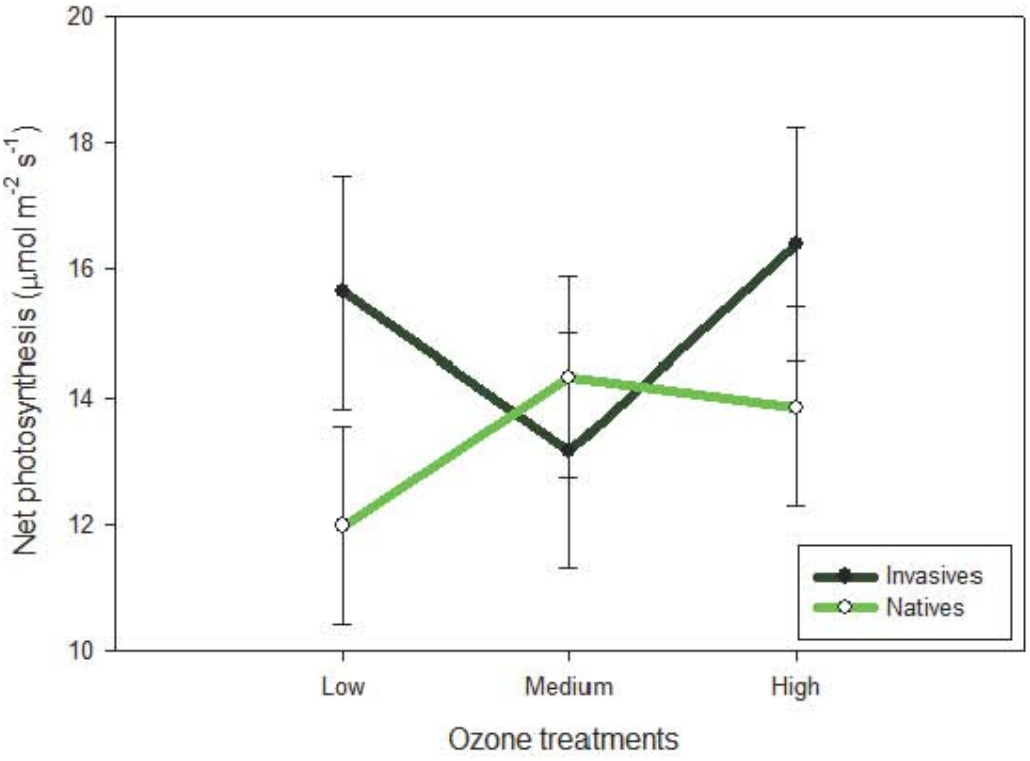

24
22
20
18
16
14
12
10
8
6
Net photosynthesis (µmol m-2 s-1)
L
M
H
Ozone treatments
AA
AP
AR
CG
CO
LT
PC
PS
PT
QAC
QAL
QR
RP

Figure 8:


20
18
16
14
12
10
Net photosynthesis (µmol m-2 s-1)
Low
Medium
High
Ozone treatments
Invasives
Natives

Appendix I: Source of saplings planted during March 2008.

Species	Source location
Acer platanoides	Cold Stream Farm, Freesoil, MI
Acer rubrum	American native plants, MD
Ailanthus altissima	Blandy Experimental Farm, VA
Carya glabra	American native plants, MD
Celtis occidentalis	American native plants, MD
Liriodendron tulipifera	Cold Stream Farm, Freesoil, MI
Paulownia tomentosa	Blandy Experimental Farm, VA
Prunus serotina	Cold Stream Farm, Freesoil, MI
Pyrus calleryana	Culley Lab, OSU, OH
Quercus acutissima	Cold Stream Farm, Freesoil, MI
Quercus alba	Cold Stream Farm, Freesoil, MI
Quercus rubra	American native plants, MD
Robinia pseudoacacia	American native plants, MD

Appendix II: Trees replaced during spring of 2009 due to browsing by white-tailed deer.

Species	# replaced
Acer platanoides	2
Ailanthus altissima	5
Carya glabra	2
Celtis occidentalis	1
Liriodendron tulipifera	1
Paulownia tomentosa	3
Prunus serotina	1
Quercus alba	1

CHAPTER 2

The role of ascorbic acid in mediating the response of native and invasive tree species to elevated ground-level ozone

Abstract

The negative effects of ground-level ozone on photosynthesis are well documented for a number of tree species. Antioxidants have been suggested as one of the primary defenses against oxidative damage caused by ozone. This study set out to examine the relationship between antioxidant levels and photosynthesis after exposure to ozone. Thirteen tree species common to the Mid-Atlantic region of the United States, including both native and invasive species, were exposed to low (20 ppb), medium (80 ppb), and high (160 ppb) ozone treatments for eight hours during the summer of 2009. Leaf extracts were prepared for constitutive (control) leaves and leaves exposed to ozone treatments to determine the constitutive and post-exposure levels of ascorbic acid for each study species. Net photosynthesis data collected during and after ozone exposures were compared with ascorbic acid levels to identify possible relationships in response to ozone treatments. Constitutive and post-exposure antioxidant levels varied among species. Native species had lower constitutive levels of ascorbic acid than invasive species, but after ozone exposures, the two groups contained similar concentrations of ascorbic acid. Overall, ozone exposure caused decreases in ascorbic acid levels. Though constitutive ascorbic acid levels did not predict ozone tolerance in terms of net photosynthesis, post-exposure ascorbic acid buffered the negative effects of ozone since the least reductions of net photosynthesis were observed in plants with high ascorbic acid levels.

Introduction

The generation of reactive oxygen species (ROS) is a natural consequence of photosynthesis. In addition to the normal production of ROS, ozone (O_3) exposure induces ROS in the apoplastic fluid and plasma membrane and readily causes damage to palisade cells (Guderian et al. 1985, Muggli et al. 1993, Rao et al. 2001). In order to combat the potentially damaging effects of ROS, leaves contain antioxidants. Accordingly, high concentrations of antioxidants have been associated with high O_3 tolerance in *Nicotiana tabacum* (Sant'Anna et al. 2008), *Medicago truncatula* (Puckette et al. 2007), and *Arabidopsis thaliana* (Conklin and Barth 2004). Antioxidants, particularly ascorbic acid (ASA), lower the observed oxidative cell damage and reduce the rate of leaf necrosis when plants are exposed to high levels (>80 ppb) of O_3 (Sant'Anna et al. 2008). The induction, reduction, and maintenance of antioxidant response as well as the sensitivity to O_3 vary among species (Wellburn and Wellburn 1996). This study addressed how levels of ASA differ among native and invasive species and investigated O_3 effects on ASA levels and net photosynthesis.

The first study to document O_3 damage to plants was initiated by Clyde Homan in 1937 at the University of Chicago. His study focused on the growth of *Phaseolus vulgaris* in ambient and O_3 enriched air, and concluded that both periodic and prolonged exposures to O_3 concentrations of 125-1000 ppb caused leaf damage. The study of O_3 effects on plants has continued since Homan's work (e.g., Haagen-Smit et al. 1952, Rich 1964, Treshow 1970, Pell and Pearson 1983, Polle et al. 1995, Matyssek et al. 2010) due in large part to the species-specific response of plants to O_3. The threshold at which

plants most frequently experience O_3 damage occurs at concentrations greater than or equal to 80 ppb (e.g., Baker et al. 1986, Bartholomay et al. 1997, Guidi et al. 1997, Emberson et al. 2000, Panek et al. 2002). Ground-level O_3 damage occurs most readily during daylight hours because the production of O_3 requires the photolysis of nitrogen dioxide (NO_2). At night, O_3 levels are lowered as high levels of nitric oxide (NO) convert O_3 into O_2 and NO_2. Leaves are the most vulnerable part of a plant to O_3 damage due to the intake of gas through stomata, the pores responsible for gas-exchange between a plant and the atmosphere (Zhang and Klessig 2001). Damage from O_3 occurs because the gas reacts with the aqueous environment of the apoplast forming reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals. These ROS denature enzymes and oxidize cell membranes resulting in cell death (Rao and Davis 2001). Ozone causes further damage to plants by decreasing carboxylation efficiency, which causes reductions in photosynthesis (Farage et al. 1991).

Recent studies have shown that plant species display a wide range of O_3 tolerance (Matyssek et al. 2008, Nagendra-Prasad et al. 2008, Agbaire 2009, Iriti and Faoro 2009, Elton Chapter 1). Plants respond to O_3 by varying above and below ground allocation and vegetative and sexual reproduction (Samuelson and Edwards 1993, Musselman et al. 1994, McLaughlin and Downing 1995, Neufeld et al. 1995, Samuelson et al. 1996). Ozone tolerance has also been associated with a reduction of stomatal conductance in a number of plant species (Reich 1987, Sharkey et al. 1996, Haikio et al. 2009). However, O_3 tolerance is most significantly linked to plant antioxidant capacity (Sharma and Davis 1997, Noctor and Foyer 1998, Smirnoff and Wheeler 2000, Pastori et al. 2003, Tausz et

al. 2007, Heath 2008). Extracellular antioxidants are the first line of defense against O_3 and provide a level of protection based on the swift (5-10 s) detoxification of ROS (Loewus 1980, Loewus 1988, Kurata et al. 1996, Smirnoff 1996, Deutsch 1997, Deutsch 1998, Sandermann et al. 1998, Asada 1999, Polle and Pell 1999). The role of antioxidants in O_3 tolerance has been studied in a large number of important crops (e.g., Cheng et al. 2007, Puckette et al. 2007, Biswas et al. 2008, Frei et al. 2008, Keutgen and Pawelzik 2008) and timber species (e.g., He et al. 2006, Then et al. 2009, Yan et al. 2010), but very little information is known about common species within communities frequently exposed to elevated ground-level O_3. Furthermore, while the role of antioxidants in O_3 tolerance has been examined for individual species, it is rarely compared among species (cf. Lee et al. 1984, Mehlhorn et al. 1986, Wellburn and Wellburn 1996, Pell et al. 1999, Severino et al. 2007). Without the standardization of experimental methods, direct comparisons of species response cannot be made. A comparison of multiple species allows for a more accurate assessment of changes that may occur in a community impacted by air pollution.

The degree of injury a plant suffers from exposure to O_3 depends on the concentrations of O_3 and antioxidants within the extracellular space. The most abundant antioxidant in the apoplast is ascorbic acid (ASA) (Polle et al. 1990, Takahama and Oniki 1992, Luwe et al. 1993, Luwe and Heber 1995, Polle et al. 1995, Kollist et al. 1996, Ranieri et al. 1996, Lyons et al. 1999a, Ranieri et al. 1999, Turcsanyi et al. 2000). Ascorbic acid can be produced in all plants through the ascorbate-glutathione cycle

(Valpuesta and Bottela 2004). Concentrations of ASA in the apoplast range from 10 μ*M* in *Fagus sylvatica* to 4000 μ*M* in *Spinacea olerace* (Lyons et al. 1999a).

In a review of 170 studies examining the role of multiple antioxidants in plant species, Lyons et al. (1999b) found ASA to be the key defense against O_3 injury when compared to peroxidases, superoxide-dismutases, polyamines, phenols, and glutathione. Though other antioxidants were found to assist with the detoxification of O_3, their contributions were minor compared to ASA. Ascorbic acid protects plants directly by reacting with ROS (Smirnoff 1996), and indirectly by reenergizing the electron donors such as alpha-tocopherol and violaxanthin in order to maintain antioxidant capacity (Packer et al. 1979, Bratt et al. 1995, Noctor and Foyer 1998). There is strong supporting evidence that ASA acts as a primary line of defense against O_3. First, the treatment of eleven populations of *Plantago* with ASA prior to O_3 exposure reduced plant injury (Zheng et al. 2000). Second, *Arabidopsis* that over-express genes in the ASA pathway protected against leaf damage after O_3 exposure (Huang et al. 2005, Eltayeb et al. 2006). *Arabidopsis* genotypes without the genes required for ASA regeneration had much greater incidence of visible leaf injury than transgenic plants.

Whether constitutive or post-exposure ASA levels are more important to the mitigation of O_3 damage is species specific. In *Phaseolus vulgairs* and *Vicia faba*, constitutive ASA levels predict O_3 damage better than levels measured after exposure to the pollutant (Burkey 1999, Turcsanyi 2000). In contrast, in *Arabadopsis*, post-exposure levels serve as a better estimate of O_3 tolerance (Pavet et al. 2005). Therefore, both

constitutive and post-exposure ASA levels should be quantified to examine their relationship to O_3 tolerance.

Ozone exposure is known to cause both reductions and inductions of ASA concentrations in plants. In some species, ASA returns to constitutive levels after O_3 exposure (Frei et al. 2008, Nagendra-Prasad et al. 2008), but most plant species have lower ASA levels after O_3 exposure (Riikonen et al. 2009, Yan et al. 2010). When examining *Ginkgo biloba*, Lu et al. (2009) found that trees maintained constitutive concentrations of ASA after exposure, signaling a restocking response of the plant without significant overall increases in ASA concentrations. Induction of ASA has been shown to be an important stress response, but this information is only available for cultivars of important agricultural crops and timber species (Frei et al. 2008, Nagendra-Prasad et al. 2008). The implications of the varied responses are unknown but may include altered competitive abilities.

The present study examined the role of ASA in controlling the photosynthetic response of a group of native and invasive tree species to elevated ground-level O_3. Ascorbic acid was chosen above all other antioxidants due to its ubiquitous nature in plants as well as its importance as the primary defensive antioxidant in plant leaves. In order to develop a full understanding of the role of ASA in the response of a plant to oxidative stress, both constitutive and post-exposure levels were quantified. The goals of this research were to: 1) determine the levels of ASA in a group of native and invasive tree species, 2) quantify how ASA levels change in response to various O_3 treatments,

and 3) determine how pre- and post-exposure levels confer resistance to the toxic effects of O_3 on net photosynthesis.

Methodology

Plant material

At the University of Virginia's Blandy Experimental Farm (Clarke County, Virginia, 39° 03' 55" N, 78° 03' 45" W) 117 two-year old saplings were planted during March of 2008. Trees were planted into a common garden to control for variation of O_3 effects caused by site conditions such as sunlight (Tausz et al. 2004), elevation (Wildi and Lutz 1996), seasonal precipitation (Garcia-Plazaola et al. 2008), and nighttime temperature (Wang and Zheng 2001). Trees were arranged into three randomized blocks aligned north to south to minimize radiation differences. Each block contained three individuals of eight native (*Acer rubrum, Carya glabra, Celtis occidentalis, Liriodendron tulipifera, Prunus serotina, Quercus alba, Quercus rubra, Robinia pseudoacacia*) and five invasive (*Acer platanoides, Ailanthus altissima, Paulownia tomentosa, Pyrus calleryana, Quercus acutissima*) species. These species include dominant-early and late successional species identified in nine urban Mid-Atlantic forests (Elton Chapter 1). Species were irrigated every day during the morning and afternoon to minimize soil moisture variability during the growing season. See Appendix I for sources of each species of tree. Nine percent of trees required replacement after the summer of 2008 due to mortality (primarily from *Odocoileus virginianus* browsing), and these are listed in Appendix II.

Experimental design and ozone fumigation

During the summer of 2009, leaves from all common garden saplings were exposed to one of three O_3 treatments. Each trial consisted of three leaf-cuvettes (Fuentes and Gillespe 1992) and three separate O_3 exposures (20 ppb, 80 ppb, 160 ppb). A sun leaf from each of three separate trees of a given species was randomly assigned an O_3 treatment and placed inside a cuvette. Trials ran from 0800 to 1600 EDT. Two peristaltic pumps (KNF Neuberger NMP850KNDC) pulled air through a system of Teflon tubing (ID 6.35 mm) connecting upstream and downstream feeds from the cuvettes to an exhaust line and a line to the gas analyzers. The flow of air through this system was regulated by a data logger (Campbell Scientific CR10) connected to two relay drivers (Campbell Scientific A21REL-12) that controlled seven 3-way solenoid valves (Asco Scientific 462312PF-12). See Elton (Chapter 1, Fig. 1) for details of the gas exchange manifold design. The first replicate of experiment began on 12 June, and the experiments ran until 28 July. Trials did not take place on days of inclement weather (8 days). The three replicates of each species were blocked into early, mid and late season trials. The order in which species were evaluated was randomized within each of these three blocks (Elton Chapter 1).

To examine post-exposure effects of O_3 on net photosynthesis an open-gas portable photosynthesis system with an infrared gas analyzer (CO750, Qubit Systems Inc., Ontario, Canada) was used one and two days after initial O_3 exposures. The Qubit leaf chamber was clamped on to each treated leaf for five minutes and measured every

other hour from 0800-1600 EDT on the first and second day after fumigation trials (Elton Chapter 1).

Forty-eight hours after O_3 treatments, control leaves (i.e., not exposed to O_3 treatments) and leaves exposed to O_3 treatments were collected and stored in a -80° C freezer at Blandy Experimental Farm. These leaves were transported to the University of Virginia's main campus (Charlottesville, VA) on dry ice, and then stored in a -80° C freezer. Twenty-five percent of leaves were damaged due to a freezer thaw, which excluded *Q. rubra* from post-exposure analysis. All other damaged samples were excluded from analyses.

Leaf extraction

To quantify ASA, leaf extracts were prepared following the methods of Nováková et al. (2008). Frozen leaves (0.20 g) were ground in pre-cooled mortars with the addition of liquid nitrogen as necessary to maintain temperature below -20°C. Ground samples were combined with 2.0 ml of pre-cooled extraction buffer (6.0% o-phosphoric acid, 2m*M* EDTA, 1% insoluble polyvinylpyrrolidone). Extracts were centrifuged for 13 minutes (24,000 g, 4 °C), and then decanted. The remaining pellet was combined with extraction buffer a second time and centrifuged. Supernatants were combined and added to 0.5 ml of the organic mobile "A buffer" (acetonitrile [HPLC grade] and 50 m*M* ammonium acetate, pH 6.8, 90:10 v/v) and filtered through a 0.45 μm filter (Puradisc Syringe Filter, Sterile). Resulting solutions were stored on ice and analyzed within 3 hours to avoid significant decreases in ASA concentrations (Nováková et al. 2008).

HILIC preparation

The protocol utilized a hydrophilic interaction liquid chromatography (HILIC) specialization of reverse phase-high performance liquid chromatography (RP-HPLC). Reversed phase-HPLC is preferable to normal phase-HPLC when sampling for small polar molecules (e.g., ASA). Hydrophilic interaction liquid chromatography is preferable to typical RP-HPLC because traditional RP-HPLC fails to reliably retain ASA (Nováková et al. 2009). The HILIC method uses a polar stationary phase and an organic mobile phase allowing for strong reproducibility, high retention, and high resolution when analyzing ASA.

An RP-HPLC machine (JASCO, Tokyo, Japan) equipped with two quaternary gradient buffer pumps (PU-2089 plus), a LC-Net II interface, and a three-line degasser (DG 2080-53) was used to run a gradient (Table 1) of mobile phase A and polar aqueous stationary phase B (70% ACN, 30% ammonium acetate) buffers. Before sample runs were queued, the RP-HPLC was thoroughly washed with HPLC-grade water (Milli-Q reverse osmosis Millipore, Medford, MA). Each of the pump lines was purged three times with 10.0 ml of water, and then the RP-HPLC was set to run at 5.0 ml per minute for 20.0 min for each line separately. After the washing of all lines in order to flush remnants from previous analyses, the flow rate was slowed to 0.1 ml per minute and the column (ZORBAX HILIC Plus, 4.6 x 100.0mm, 3.5 μm, Santa Clara, CA) was attached. The column was then washed for 30.0 min at a flow rate of 1.0 ml per minute. After the column was washed with water, the column was sealed and removed while the same steps were repeated after the addition of the aqueous and organic buffers. Following the

washing of the RP-HPLC with the buffers, the column was attached and equilibrated in a ratio of 90:10 (organic: aqueous) for 30 min at 1.0 ml per minute before the first sample run.

Sample runs

After the leaf extraction process, 20.0 μl of a sample were injected (Rheodyne 7125, Berkeley, CA) into a 10.0 μl sample loop. Each sample was run with four replicates, and between each run the sample loop was washed with 60.0 μl of buffer A to prevent residual sample constituents contaminating future runs. For each sample, one replicate included the addition of quantified ASA standard (Sigma Aldrich C3878). Each HILIC run lasted 20.0 min at a flow rate of 0.5 ml per minute including a column equilibration period of 10.0 min before each new run. The gradient of buffers A and B (Table 1) was designed to flush the hydrophobic compounds contained in the leaf extract early in the run, leaving the hydrophilic ASA on the column to elute later at higher clarity and resolution, which was verified using a mass spectrometer (Thermo-Finnigan SSQ7000). Ascorbic acid eluted from the column at 14.0 min (Fig. 1) and absorbance was determined with a UV detector (JASCO UV-2075 plus) set for 268 nm (Nováková et al. 2008). Data acquisition and quantification were performed with Chrompass software (JASCO) using the calibration curve (0.05-500.00 $\mu g\ ml^{-1}$, $y = 51329x - 579592$, $R^2 = 0.9980$) of authentic ASA (Sigma Aldrich A5960).

Accuracy and precision

Standardized validation methods were used (Nováková et al. 2008) following International Conference on Harmonization of Technical Requirements. The use of an internal standard required a reference standard that was a polar acidic compound due to the use of the HILIC separation technique. Chlorogenic acid was determined to perform best among a group of several phenolic acids (e.g., gallic acid, protocatechuic acid) as it eluted well before ASA (Nováková et al. 2008). The inclusion of this internal standard with a single run from each sample allowed for the observation of potential shifts in the curve. When these shifts were observed the RP-HPLC was rewashed and samples were repeated. Samples were also processed with a spectrophotometer (Varian, Cary 100 Bio UV/Visible, Palo Alto, CA) to test for UV spectra accuracy.

Statistical analyses

To test the hypothesis that species vary in constitutive antioxidant levels, ASA levels in leaves that had not been exposed to O_3 treatments were compared across species. An ANOVA was used with the SAS PROC MIXED (version 9.1.3; SAS Institute, Cary, NC, USA) that included status (native or invasive) of each species as a fixed effect. Random effects in the ANOVA model included species nested within status and block. When necessary, additional ANOVAs were performed to calculate log-likelihood ratio tests (G) to measure the significance of random effects (Littell et al. 2006).

A repeated measures ANOVA was performed to test for differences between constitutive and post-exposure levels for the low, medium, and high O_3 treatments. Independent fixed effects included O_3 treatments, native/invasive species status, time, O_3*status interaction, and O_3*time interaction. Independent random variables included species and species*O_3 interaction. The unit of replication was tree nested within species*O_3 treatment interaction. Contrasts were performed for comparison of ASA levels between day one and day two for low, medium, and high O_3 treatments. Only those species for which data was available for multiple replicates were included.

To examine the relationship between ASA and net photosynthesis after O_3 treatment, ANOVAs were performed for both constitutive and post-exposure levels of ASA. The independent variables in this model included fixed effects of ASA levels, O_3 treatment (excluding low treatment), and ASA*O_3 treatment interaction while species and a species*O_3 treatment interaction were modeled as random effects.

Results

Constitutive ascorbic acid

Three species (*C. occidentalis*, *P. calleryana*, *R. pseudoacacia*) had undetectable levels (<0.50 μmol/g FW) of ASA and were excluded from analyses. Species varied significantly in constitutive ASA levels (Table 2), ranging from 0.79±0.32 μmol/g FW (*L. tulipifera*, ± indicates 1 standard error) to 5.91±0.54 μmol/g FW (*A. altissima*) (Fig. 2). A Tukey-Kramer test of means grouped species into three categories (Fig. 2).

On average, invasive species exhibited significantly higher levels of constitutive ASA than native species (Table 2), a 2.7 fold difference. The comparison of native and invasive species in this study did not have phylogenetic control. The differences therefore cannot be assumed to occur for all native and invasive species comparisons.

Constitutive versus post-exposure ascorbic acid

A repeated measures ANOVA showed a significant O_3 treatment*time interaction indicating that ASA levels changed significantly after O_3 exposure in some of the O_3 treatments (Table 3, Fig. 4). A-priori contrasts showed no changes in ASA when comparing the constitutive and low O_3 treatment leaves (t=0.44, df=18, p=0.6641) indicating that low treatments did not impact the production of ASA. The largest difference (-65.95%) between constitutive and post-exposure ASA levels occurred in the high treatment (t=5.10, df=18, p<0.0001) while medium treatments also caused a significant decrease (-59.62%) in ASA levels (t=3.48, df =18, p=0.0018). Species varied significantly in post-exposure ASA levels (Table 3, Fig. 5). The highest levels were from *P. serotina* (6.60±0.59 μmol/g FW) while *P. tomentosa* contained the lowest levels (0.67±0.13 μmol/g FW). Native and invasive species did not differ in ASA level after O_3 treatments (Table 3), which contrasts with the differences noted in constitutive ASA levels.

Constitutive ascorbic acid and the change of net photosynthesis

The change in net photosynthesis two days after O_3 exposure trials was not linked to constitutive ASA concentrations (Table 5, Fig. 6). Increased exposure to elevated O_3 in the medium and high O_3 treatments caused a decrease in net photosynthesis two days after O_3 exposure. The change in net photosynthesis varied by species (Table 5). These results indicate that constitutive ASA levels do not serve as a good predictor of O_3 tolerance as measured by the change of net photosynthesis.

Post-exposure ascorbic acid and the change of net photosynthesis

The levels of ASA measured two days after O_3 exposure trials had a significant positive effect on the change of net photosynthesis (Table 6, Fig. 7). Plants with low levels of post-exposure ASA suffered reductions in net photosynthesis. Increases or maintenance of net photosynthesis were noted in plants with high post-exposure ASA. Post-exposure ASA provided a better estimate of O_3 tolerance than constitutive ASA levels.

Discussion

Constitutive ascorbic acid

The tree species in this study varied in concentrations of ASA before exposure to elevated O_3. The closely related congeners of *Acer* contained similar concentrations of ASA, but in the genus *Quercus,* the invasive *Q. acutissima* had significantly higher ASA levels than the native *Q. alba* and *Q. rubra*, suggesting that phylogeny is not the only

driver that influences ASA concentrations. The *Acer* spp. contained some of the highest concentrations of constitutive ASA, while *Carya* and the native *Quercus* spp. were among the species with the lowest concentrations of ASA. Most Mid-Atlantic forests are *Acer* spp. or *Carya*/*Quercus* spp. dominated (Braun 1950, Smith et al. 2003, Pan et al. 2010), so these differences in constitutive ASA may indicate differences between these two communities in initial sensitivity to oxidative stress. Environmental stresses can select for new community assemblages that favor stress-tolerant species. For example, areas in northeastern France with polluted soils have plant communities comprising species with higher levels of antioxidants than those occupying unpolluted soils (Dazy et al. 2009). However, plants may adapt to stress by increasing antioxidant capacity, as seen in the rainforest of Barro Colorado Island, Panama (Frankel 1999). The majority of species found in areas of high oxidative stress had more antioxidants compared to plants in low stress environments (Frankel 1999). To fully evaluate the role of antioxidants in stress response, it is essential to examine antioxidants after exposure to stress events.

Invasive species contained higher constitutive levels of ASA than native species. This may indicate a potential defense against stressful environments which non-invasive species lack. Although ASA has been identified as a defense against oxidative stress and a precursor to other plant defense chemicals (An et al. 2009; cf. Ralph 2009), it plays a broader role in preventing damage from environmental stressors such as drought (Reddy et al. 2004), temperature (Iba 2002), and photoperiod (Mittler 2002). The defense strategies of plants are complex and interrelated, so it is likely that the high level of ASA produced by invasive species provides broad protection against environmental stresses.

The majority of invasive species typify the stress-tolerant plant strategy (Di Tomaso 1998, Cavas and Yurdak 2005, Maskell et al. 2006) described by Grime (1977). High concentrations of ASA may enable invasiveness by protecting against cumulative negative effects of various stresses, as was found with an invasive alga (*Caulerpa racemosa*) in the Mediterranean Sea (Cavas and Yurdak 2005). It is possible that the high levels of ASA found among invasive tree species in this study are an artifact of taxon sampling. Future studies should expand this assessment to other invasive and native tree species.

Post-exposure ascorbic acid

Trees exposed to increased oxidative stress in the medium and high treatments sustained reductions in ASA, which presumably occurred due to the reaction of ROS with ASA at a rate that exceeded the rate of ASA replenishment (Frei et al. 2008, Esposito et al. 2009, Singh et al. 2010). Species varied in post-exposure ASA concentrations. The magnitude of ASA reduction of a species depends on the ability of a species to replenish the ASA pool, which has been attributed to genetic controls that vary among species (Frei et al. 2008).

Decreases in levels of ASA due to its role as an antioxidant may have cascading effects by altering other plant functions that require ASA. Reductions of ASA in plant leaves caused by O_3 could form a positive feedback loop by promoting premature senescence (Navabpour et al., 2007), which would then cause further reductions of ASA in leaves unaffected by O_3 through abscisic acid hormone signaling. Decreases in ASA

concentration may also impact guard cell regulation (Chen and Gallie 2004), enzyme cofactor activity (Davey et al. 2000), and cell proliferation and elongation (Smirnoff 1996).

Native and invasive species contained similar concentrations of ASA after O_3 exposures. Both groups of species suffered reductions of ASA in response to oxidative stress, but the invasive species incurred greater losses effectively balancing ASA defense between native and invasive species.

Constitutive ascorbic acid and the change of net photosynthesis

The change in net photosynthesis after O_3 exposure trials was used as a proxy for O_3 tolerance (Barnes et al. 1999, Barnes et al. 2000). In this study, constitutive ASA concentrations in tree leaves did not predict O_3 tolerance. These results contrast with past findings that suggest plants invest in constitutive ASA levels as a more effective means of defending against oxidative damage compared to the replenishment and transport of ASA after oxidative stress (Burkey et al. 2000, Robinson and Britz 2000, Pasqualini et al. 2002). Since constitutive ASA levels did not influence O_3 tolerance in the tree species, it is likely that these species do not preemptively protect against high levels of oxidative stress. More recent studies (Baek and Woo 2010, Bulbovas et al. 2010, Pina and Moraes 2010) have demonstrated that high concentrations of antioxidants in trees does not forestall oxidative damage, as was demonstrated in the present study. Plants have evolved antioxidant systems to deal with oxidative stress that we find in many clean air environments. However, due to the rapid increase in oxidant air pollution, long-lived

trees have not been afforded the necessary time between generations to adapt to such change. Plants can respond effectively to moderate levels of oxidative stress by inducing antioxidant production, but such inductions do not stave off damage when pollution exceeds levels for which plants are adapted.

Post-exposure ascorbic acid and the change of net photosynthesis

After O_3 treatments, it was clear that individuals with the highest ASA concentrations also maintained the highest values of net photosynthesis. This pattern supports the known role of ASA as an important antioxidant detoxifier of O_3 in plants (Smirnoff 1996, Frei et al. 2008, Nagendra-Prasad et al. 2008). Photosynthetic rates in plants with high levels of ASA are expected to maintain higher rates when exposed to multiple O_3 events. Assuming that these trends continue throughout a growing season (Herbinger et al. 2005), species able to induce ASA after O_3 exposure would have a growth advantage over those that sustain reductions in ASA.

The differences in post-exposure ASA among species may account for differences in O_3 tolerance (Lee et al. 1984, Calatayud et al. 2002, 2003, Haberer et al. 2006, Severino et al. 2007). When examining the effects of O_3 on O_3 tolerant and O_3 sensitive clones of *Trifolium repens*, the post-exposure ASA concentrations for the tolerant clone were 50-70% higher than in the sensitive clone (Severino et al. 2007). Strong inverse relationships between visible leaf injury and post-exposure ASA concentrations are often found (Severino et al. 2007, Esposito et al. 2009, Pina and Moraes 2010), further supporting the role of post-exposure ASA as a predictor of O_3 tolerance in plants.

The increases in net photosynthesis seen in plants with high post-exposure ASA concentrations were much greater than expected. Because photosynthetic measurements were taken with different instruments during O_3 exposure and two days after O_3 exposure, it is possible that the seemingly elevated photosynthetic rates could be a methodological artifact. However, a comparison of photosynthetic rates in the low (ambient) treatment found no significant difference between rates during and two days after exposure (Elton Chapter 1). This suggests that the elevated photosynthetic rates are not simply artifacts.

One possible explanation for the elevated photosynthetic rates two days after O_3 exposure is the bolstering of a type of photorespiration called the Mehler peroxidase reaction (MPR), for which ASA is an enzyme cofactor. In non-stressful environments MPR contributes very little to photorespiration (Badger et al. 2000), but it plays an important role in stressful environments, allowing for cyclic electron transport and greater production of ATP (Ivanov and Edwards 1997). *Prunus serotina*, which exhibited an increase in ASA levels after O_3 exposure, may have had higher net photosynthesis as a result of the strengthening of MPR. Rice plants that utilize MPR under stressful conditions have shown a 20% increase in carbon assimilation (as measured by CO_2 flux) when compared to control plants (Makino et al 2002). By increasing the production of ATP and NADPH, MPR allows for higher consumption of carbon dioxide, which can lead to temporary increases in net photosynthesis.

The strong relationship between high post-exposure ASA concentrations and an overall increase in net photosynthesis is due to the trends of two species. Both *P.*

serotina and *A. rubrum* showed marked increases in net photosynthesis after exposure to O_3 treatments, while the majority of species showed much more subtle changes in net photosynthesis. *P. serotina* had increases in ASA levels after O_3 exposures while *Acer rubrum* maintained ASA levels. Therefore, enhanced performance of both *P. serotina* and *A. rubrum* may be expected in forests experiencing elevated air pollution.

Conclusions

The results from this study suggest that ASA can play a significant role in buffering the negative effects of O_3 stress on gas-exchange processes. Importantly, ASA levels measured after O_3 exposure were a better predictor of O_3 tolerance than constitutive levels. Constitutive ASA levels varied among species, but similarities were not determined entirely by phylogentic relatedness. Invasive species showed a potential competitive advantage over native species by containing higher levels of constitutive ASA, but this may have been a result of the specific species used in this study and not necessarily a general trend expected between other native and invasive species.

After exposure to O_3, the mean ASA concentrations dropped, but to varying degrees such that species still significantly differed in ASA levels. The trees capable of maintaining the highest levels of ASA after O_3 exposure exhibited the lowest reductions and sometimes increases in net photosynthesis. Differences in post-exposure ASA levels among species help to explain the O_3 tolerance of plants.

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Literature Cited

Agbaire, P O. 2009. Air pollution tolerance indices (APTI) of some plants around Erhoike-kokori oil exploration site of delta state, Nigeria. *International Journal of Physical Sciences* 4: 366-368.

An, Y, Y Shen, and Z Zhang. 2009. Effects of mechanical damage and herbivore wounding on H_2O_2 metabolism and antioxidant enzyme activities in hybrid poplar leaves. *Journal of Forestry Research* 20: 156-160.

Asada, K. 1999. The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 601-639.

Badger, M R, S von Caemmerer, S Ruuska, and H Nakano. 2000. Electron flow to oxygen in higher plants and algae: rates and control of direct photoreduction (Mehler reaction) and rubisco oxygenase. *Philosophical Transactions of The Royal Society B: Biological Sciences* 355: 1433-1446.

Baek, S G and S Y Woo. 2010. Physiological and biochemical responses of two tree species in urban areas to different air pollution levels. *Photosynthetica* 48: 23-29.

Baker, C K, J J Colls, A E Fullwood, and G G R Seaton. 1986. Depression of growth and yield in winter barley exposed to sulphur dioxide in the field. *New Phytologist* 104: 233-241.

Barnes, J D, T Lyons, J Maddison, E Turcsanyi, Y Zheng, and M Plöchl. 2000. Extracellular ascorbate: A potential first-line of defence against ozone. *Global Environmental Researches on Biology and Ecological Aspects* 1: 55-66.

Barnes, J D, J Bender, T Lyons, and A Borland. 1999. Natural and man-made selection for air pollution resistance. *Journal of Experimental Botany* 50: 1423.

Bartholomay, G A, R T Eckert, and K T Smith. 1997. Reductions in tree-ring widths of white pine following ozone exposure at Acadia National Park, Maine, USA. *Canadian Journal of Forest Research* 27: 361-368.

Bernardi, R, C Nali, P Ginestri, C Pugliesi, G Lorenzini, and M Durante. 2004. Antioxidant enzyme isoforms on gels in two poplar clones differing in sensitivity after exposure to ozone. *Biologia Plantarum* 48: 41-48.

Biswas, D K, H Xu, Y G Li, J Z Sun, X Z Wang, X G Han, and G M Jiang. 2008. Genotypic differences in leaf biochemical, physiological and growth responses to ozone in 20 winter wheat cultivars released over the past 60 years. *Global Change Biology* 14: 46-59.

Bratt, C E, P O Arvidsson, M Carlsson, and H E Åkerlund. 1995. Regulation of violaxanthin de-epoxidase activity by ph and ascorbate concentration. *Photosynthesis Research* 45: 169-175.

Braun, E L. 1950. Deciduous forests of the eastern United States. In *Hafner Publ. Co.* New York. NY 596P.

Bulbovas, P, R M de Moraes, M C S Rinaldi, A L Cunha, W B C Delitti, and M Domingos. 2010. Leaf antioxidant fluctuations and growth responses in saplings of *Caesalpinia echinata* lam. (Brazilwood) under an urban stressing environment. *Ecotoxicology and Environmental Safety* 73: 664-670.

Burkey, K O, C Wei, G Eason, P Ghosh, and G P Fenner. 2000. Antioxidant metabolite levels in ozone-sensitive and tolerant genotypes of snap bean. *Physiologia Plantarum* 110: 195-200.

Burkey, K O. 1999. Effects of ozone on apoplast/cytoplasm partitioning of ascorbic acid in snap bean. *Physiologia Plantarum* 107: 188-193.

Calatayud, A, D J Iglesias, M Talón, and E Barreno. 2003. Effects of 2-month ozone exposure in spinach leaves on photosynthesis, antioxidant systems and lipid peroxidation. *Plant Physiology and Biochemistry* 41: 839-845.

Calatayud, A, J W Alvarado, and E Barreno. 2002. Differences in ozone sensitivity in three varieties of cabbage (*Brassica oleracea* L.) in the rural Mediterranean area. *Journal of Plant Physiology* 159: 863-868.

Cavas, L and K Yurdakoc. 2005. A comparative study: Assessment of the antioxidant system in the invasive green alga *caulerpa racemosa* and some macrophytes from the Mediterranean. *Journal of Experimental Marine Biology and Ecology* 321: 35-41.

Chen, Z and D R Gallie. 2004. The ascorbic acid redox state controls guard cell signaling and stomatal movement. *The Plant Cell* 16: 1143-1162.

Cheng, F-Y, K O Burkey, J M Robinson, and F L Booker. 2007. Leaf extracellular ascorbate in relation to O_3 tolerance of two soybean cultivars. *Environmental Pollution* 150: 355-62.

Conklin, P L and C Barth. 2004. Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. *Plant, Cell and Environment* 27: 959-970.

Davey, M W, M V Montagu, D Inze, M Sanmartin, A Kanellis, N Smirnoff, I J J Benzie, J J Strain, D Favell, and J Fletcher. 2000. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects on processing. *Journal of the Science of Food and Agriculture* 80: 825-860.

Dazy, M, E Béraud, S Cotelle, F Grévilliot, J F Férard, and J F Masfaraud. 2009. Changes in plant communities along soil pollution gradients: Responses of leaf antioxidant enzyme activities and phytochelatin contents. *Chemosphere* 77: 376-383.

Degl'Innocenti, E, C Vaccę, L Guidi, and G F Soldatini. 2003. CO_2 photoassimilation and chlorophyll fluorescence in two clover species showing different response to O_3. *Plant Physiology and Biochemistry* 41: 485-493.

Deutsch, J C. 1998. Ascorbic acid oxidation by hydrogen peroxide. *Analytical Biochemistry* 255: 1-7.

Deutsch, J C. 1997. Ascorbic acid and dehydroascorbic acid interconversion without net oxidation or reduction. *Analytical Biochemistry* 247: 58-62.

Di Tomaso, J M. 1998. Impact, biology, and ecology of salt cedar (*Tamarix* spp.) In the southwestern United States. *Weed Technology* 12: 326-336.

Eltayeb, A E, N Kawano, G H Badawi, H Kaminaka, T Sanekata, I Morishima, T Shibahara, S Inanaga, and K Tanaka. 2006. Enhanced tolerance to ozone and drought stresses in transgenic tobacco overexpressing dehydroascorbate reductase in cytosol. *Physiologia Plantarum* 127: 57-65.

Emberson, LD, G Wieser, and MR Ashmore. 2000. Modelling of stomatal conductance and ozone flux of Norway spruce: Comparison with field data. *Environmental Pollution*, 19: 393-402.

Esposito, M P, M L Ferreira, S M R Sant'Anna, M Domingos, and S R Souza. 2009. Relationship between leaf antioxidants and ozone injury in *Nicotiana tabacum* 'bel-w3' under environmental conditions in Sao Paulo, SE - Brazil. *Atmos Environmental* 43: 619-623.

Farage, P K, S P Long, E G Lechner, and N R Baker. 1991. The sequence of change within the photosynthetic apparatus of wheat following short-term exposure to ozone. *Plant Physiology* 95: 529.

Frankel, S and M Berenbaum. 1999. Effects of light regime on antioxidant content of foliage in a tropical forest community. *Biotropica* 31: 422-429.

Frei, M, J P Tanaka, and M Wissuwa. 2008. Genotypic variation in tolerance to elevated ozone in rice: Dissection of distinct genetic factors linked to tolerance mechanisms. *Journal of Experimental Botany* 59: 3741-52.

Fuentes, J D and T J Gillespie. 1992. A gas exchange system to study the effects of leaf surface wetness on the deposition of ozone. *Atmospheric Environment. Part A, General Topics* 26: 1165-1173.

Garcia-Plazaola, J I, R Esteban, K Hormaetxe, B Fernandez-Marin, and J M Becerril. 2008. Photoprotective responses of Mediterranean and Atlantic trees to the extreme heat-wave of summer 2003 in southwestern Europe. *Trees-Structure and Function* 22: 385-392.

Grime, J P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111: 1169-1194.

Guderian, R, D T Tingey, and R Rabe. 1985. *Air Pollution by Photochemical Oxidants. Formation, Transport, Control, and Effects on Plants.* Springer-Verlag

Guidi, L, C Nali, G Lorenzini, F Filippi, and G F Soldatini. 2001. Effect of chronic ozone fumigation on the photosynthetic process of poplar clones showing different sensitivity. *Environmental Pollution* 113: 245-254.

Guidi, L, C Nali, S Ciompi, G Lorenzini, and G F Soldatini. 1997. The use of chlorophyll fluorescence and leaf gas exchange as methods for studying the different responses to ozone of two bean cultivars. *Journal of Experimental Botany* 48: 173.

Haagen-Smit, A J, E F Darley, M Zaitlin, H Hull, and W Noble. 1952. Investigation on injury to plants from air pollution in the Los Angeles area. *Plant Physiology* 27: 18.

Haberer, K, L Jaeger, and H Rennenberg. 2006. Seasonal patterns of ascorbate in the needles of Scots pine (*Pinus sylvestris* L.) Trees: Correlation analyses with atmospheric O_3 and NO_2 gas mixing ratios and meteorological parameters. *Environmental Pollution* 139: 224-31.

Haikio, E, M Makkonen, R Julkunen-Tiitto, J Sitte, V Freiwald, T Silfver, V Pandey, E Beuker, T Holopainen, and E Oksanen. 2009. Performance and secondary chemistry of two hybrid aspen (*Populus tremula* L. X *Populus tremuloides* michx.) Clones in long-term elevated ozone exposure. *Journal of Chemical Ecology* 35: 664-678.

He, X Y, Y N Ruan, W Chen, and T Lu. 2006. Responses of anti-oxidative system in leaves of *Ginkgo biloba* to elevated ozone concentration in urban area. *Botanical Studies* 47: 409-416.

Heath, R L. 2008. Modification of the biochemical pathways of plants induced by ozone: What are the varied routes to change? *Environmental Pollution* 155: 453-63.

Herbinger, K, Ch Then, M Löw, K Haberer, M Alexous, N Koch, and K Remele. 2005. Tree age dependence and within-canopy variation of leaf gas exchange and antioxidative defence in *Fagus sylvatica* under experimental free-air ozone exposure. *Environmental Pollution* 137: 476-82.

Homan, C. 1937. Effects of ionized air and ozone on plants. *Plant Physiology* 12: 957.

Huang, D, B Ou, and R L Prior. 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry* 53: 1841-1856.

Iba, K. 2002. Acclimative response to temperature stress in higher plants: Approaches of gene engineering for temperature tolerance. *Plant Biology* 53: 225.

Iriti, M and F Faoro. 2009. Chemical diversity and defence metabolism: How plants cope with pathogens and ozone pollution. *International Journal of Molecular Sciences* 10: 3371-99.

Ivanov, B, and G E, Edwards. 1997. Electron flow accompanying the ascorbate peroxidase cycle in maize mesophyll chloroplasts and its cooperation with linear electron flow to NADP+ and cyclic electron flow in thylakoid membrane energization. *Photosynthesis Research* 52: 187-198.

Keutgen, A J and E Pawelzik. 2008. Apoplastic antioxidative system responses to ozone stress in strawberry leaves. *Journal of Plant Physiology* 165: 868-75.

Kollist, H, H Moldau, L Mortensen, S K Rasmussen, and L B Jørgensen. 1996. Ascorbate levels and ozone decay in the cell walls of barley and wheat seedlings. In *Plant Peroxidases: Biochemistry and Physiology. IV. International Symposium, Vienna.*

Kurata, T, N Miyake, and Y Otsuka. 1996. Formation of l-threonolactone and oxalic acid in the autoxidation reaction of l-ascorbic acid: Possible involvement of single oxygen. *Bioscience, Biotechnology, and Biochemistry* 60: 1212-1214.

Lee, E H, J A Jersey, C Gifford, and J Bennett. 1984. Differential ozone tolerance in soybean and snapbeans: Analysis of ascorbic acid in O_3-susceptible and O_3-resistant cultivars by high-performance liquid chromatography. *Environmental and Experimental Botany* 24: 331-335.

Littell, R C. 2006. *SAS for Mixed Models.* SAS Publishing.

Loewus, F A. 1988. Ascorbic acid and its metabolic products. *The Biochemistry of Plants: A Comprehensive Treatise* 14.

Loewus, F A. 1980. L-Ascorbic acid: Metabolism, biosynthesis, function. *The Biochemistry of Plants: A Comprehensive Treatise (USA)*.

Lu, T, X He, W Chen, K Yan, and T Zhao. 2009. Effects of elevated O_3 and/or elevated CO_2 on lipid peroxidation and antioxidant systems in *Ginkgo biloba* leaves. *Bulletin of Environmental Contamination and Toxicology* 83: 92-6.

Luwe, M and U Heber. 1995. Ozone detoxification in the apoplasm and symplasm of spinach, broad bean and beech leaves at ambient and elevated concentrations of ozone in air. *Planta* 197: 448-455.

Luwe, M W F, U Takahama, and U Heber. 1993. Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia oleracea* L.) Leaves. *Plant Physiology* 101: 969.

Lyons, T, J H Ollerenshaw, and J D Barnes. 1999a. Impacts of ozone on *Plantago major*: Apoplastic and symplastic antioxidant status. *New Phytologist* 141: 253-263.

Lyons, T, M Plochl, E Turcsanyi, and J Barnes. 1999b. Extracellular antioxidants: A protective screen against ozone. *Environmental Pollution and Plant Responses.* 183-201.

Marabottini, R, C Schraml, A R Paolacci, A Sorgona, A Raschi, H Rennenberg, and M Badiani. 2001. Foliar antioxidant status of adult Mediterranean oak species (*Quercus ilex* L. And *Q. pubescens* willd.) Exposed to permanent CO_2-enrichment and to seasonal water stress. *Environmental Pollution* 115: 413-23.

Maskell, L C, J M Bullock, S M Smart, K Thompson, and P E Hulme. 2006. The distribution and habitat associations of non-native plant species in urban riparian habitats. *Journal of Vegetation Science* 17: 499-508.

Matyssek, R, D F Karnosky, G Wieser, K Percy, E Oksanen, T E E Grams, M Kubiske, D Hanke, and H Pretzsch. 2010. Advances in understanding ozone impact on forest trees: Messages from novel phytotron and free-air fumigation studies. *Environmental Pollution* 158: 1990-2006.

Matyssek, R, H Sandermann, G Wieser, F Booker, S Cieslik, R Musselman, and D Ernst. 2008. The challenge of making ozone risk assessment for forest trees more mechanistic. *Environmental Pollution* 156: 567-82.

McLaughlin, S B and D J Downing. 1995. Interactive effects of ambient ozone and climate measured on growth of mature forest trees. *Nature* 374 (6519): 252-254.

Mehlhorn, H, G Seufert, A Schmidt, and K J Kunert. 1986. Effect of SO_2 and O_3 on production of antioxidants in conifers. *Plant Physiology* 82: 336.

Makino, A, C Miyake, and A Yokota. 2002. Physiological functions of the water-water cycle (Mehler Reaction) and the cyclic electron flower around PSI in rice leaves. *Plant Cell Physiology* 43: 1017-1026

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* 7: 405-410.

Muggli. T. 1993. *Free Radical Tissue Damage: The Protective Role of Antioxidants.* Ed. Corongiu, Banni, Dessi, and Rice-Evans. London: Richelieu Press.

Musselman, R C, T Younglove, and P M McCool. 1994. Response of *Phaseolus vulgaris* L. to differing ozone regimes having identical total exposure. *Atmospheric Environment* 28: 2727-2731.

Nagendra-Prasad, D, N Sudhakar, K Murugesan, and N Mohan. 2008. Pre-Exposure of calli to ozone promotes tolerance of regenerated *Lycopersicon esculentum* cv. PKM1 plantlets against acute ozone stress. *Journal of Plant Physiology* 165: 1288-99.

Nali, C, L Guidi, F Filippi, G F Soldatini, and G Lorenzini. 1998. Photosynthesis of two poplar clones contrasting in O_3 sensitivity. *Trees-Structure and Function* 12: 196-200.

Navabpour, S, M B Bagherieh-Najjar, and H Soltanloo. 2007. Identification of novel genes expressed in *Brassica napus* during leaf senescence and in response to oxidative stress. *International Journal of Plant Production* 1:1.

Neufeld, H S, E H Lee, J R Renfro, W D Hacker, and B H Yu. 1995. Sensitivity of seedlings of black cherry (*Prunus serotina* ehrh.) To ozone in Great Smoky Mountains National Park. I. Exposure-Response curves for biomass. *New Phytologist* 130: 447-459.

Noctor, G and C H Foyer. 1998. Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review of Plant Biology* 49: 249-279.

Nováková, L, D Solichová, and P Solich. 2009. Hydrophilic interaction liquid chromatography - charged aerosol detection as a straightforward solution for simultaneous analysis of ascorbic acid and dehydroascorbic acid. *Journal of Chromatography* 1216: 4574 - 4581.

Nováková, L, D Solichová, S Pavlovicová, and P Solich. 2008. Hydrophilic interaction liquid chromatography method for the determination of ascorbic acid. *Journal of Separation Science* 31: 1634-44.

Packer, J E, T F Slater, and R L Willson. 1979. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* 278: 737-738.

Pan, Y, J M Chen, R Birdsey, K McCullough, L He, F Deng, and F S USDA. 2010. Age structure and disturbance legacy of north American forests. *Biogeosciences Discussion* 7, 979-1020.

Panek, J A, M R Kurpius, and A H Goldstein. 2002. An evaluation of ozone exposure metrics for a seasonally drought-stressed ponderosa pine ecosystem. *Environmental Pollution* 117: 93-100.

Pasqualini, S, M Antonielli, L Ederli, C Piccioni, and F Loreto. 2002. Ozone uptake and its effect on photosynthetic parameters of two tobacco cultivars with contrasting ozone sensitivity. *Plant Physiology and Biochemistry* 40: 599-603.

Pastori, G M, G Kiddle, J Antoniw, S Bernard, S Veljovic-Jovanovic, P J Verrier, G Noctor, and C H Foyer. 2003. Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. *Plant Cell* 15: 939-51.

Pavet, V, E Olmos, G Kiddle, S Mowla, S Kumar, J Antoniw, M E Alvarez, and C H Foyer. 2005. Ascorbic acid deficiency activates cell death and disease resistance responses in *Arabidopsis*. *Plant Physiol*ogy 139: 1291.

Pell, E J, J P Sinn, B W Brendley, L Samuelson, C Vinten-Johansen, M Tien, and J Skillman. 1999. Differential response of four tree species to ozone-induced acceleration of foliar senescence. *Plant Cell and Environment* 22: 779-790.

Pell, E J and N S Pearson. 1983. Ozone-Induced reduction in quantity of ribulose-1,5-bisphosphate carboxylase in alfalfa foliage. *Plant Physiology* 73: 185-187.

Pina, J M and R M Moraes. 2010. Gas exchange, antioxidants and foliar injuries in saplings of a tropical woody species exposed to ozone. *Ecotoxicology and Environmental Safety* 73: 685-691.

Polle, A K and E J Pell. 1999. Role of carbon dioxide in modifying the plant response to ozone. In *Carbon Dioxide and Environmental Stress.* Academic Press.

Polle, A, G Wieser, and WM Havranek. 1995. Quantification of ozone influx and apoplastie ascorbate content in needles of Norway spruce trees (*Picea abies* L., Karst) at high altitude. *Plant, Cell and Environment* 18: 681-688.

Polle, A, K Chakrabarti, W Schurmann, and H Renneberg. 1990. Composition and properties of hydrogen peroxide decomposing systems in extracellular and total extracts from needles of Norway spruce (*Picea abies* L., Karst.). *Plant Physiology* 94: 312.

Puckette, M C, H Weng, and R Mahalingam. 2007. Physiological and biochemical responses to acute ozone-induced oxidative stress in *Medicago truncatula*. *Plant Physiology and Biochemistry* 45: 70 - 79.

Ralph, S G. 2009. Studying *Populus* defenses against insect herbivores in the post-genomic era. *Critical Reviews in Plant Sciences* 28: 335-345.

Ranieri, A, A Castagna, E Padu, H Moldau, M Rahi, and G F Soldatini. 1999. The decay of O_3 through direct reaction with cell wall ascorbate is not sufficient to explain the different degrees of O_3-sensitivity in two poplar clones. *Journal of Plant Physiology* 154: 250-255.

Ranieri, A, G D'urso, C Nali, G Lorenzini, and G F Soldatini. 1996. Ozone stimulates apoplastic antioxidant systems in pumpkin leaves. *Physiologia Plantarum* 97: 381-387.

Rao, M V and K R Davis. 2001. The physiology of ozone induced cell death. *Planta* 213: 682-690.

Reddy, A R, K V Chaitanya, and M Vivekanandan. 2004. Drought-Induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology* 161: 1189-1202.

Reich, P B. 1987. Quantifying plant response to ozone: A unifying theory. *Tree Physiology* 3: 63-91.

Rich, S. 1964. Ozone damage to plants. *Annual Review of Phytopathology* 2: 253-266.

Riikonen, J, N Mäenpää, N Alavillamo, T Silfver, and E Oksanen. 2009. Interactive effect of elevated temperature and O3 on antioxidant capacity and gas exchange in *Betula pendula* saplings. *Planta* 230: 419-27.

Robinson, J and S J Britz. 2000. Tolerance of a field grown soybean cultivar to elevated ozone level is concurrent with higher leaflet ascorbic acid level, higher ascorbate-dehydroascorbate redox status, and long term photosynthetic productivity. *Photosynthesis Research* 64: 77-87.

Samuelson, L J, J M Kelly, P A Mays, and G S Edwards. 1996. Growth and nutrition of Quercus rubra L. Seedlings and mature trees after three seasons of ozone exposure. *Environmental Pollution* 91: 317-23.

Samuelson, L J and G S Edwards. 1993. A comparison of sensitivity to ozone in seedlings and trees of *Quercus rubra* L. *New Phytologist* 125: 373-379.

Sandermann, H, D Ernst, W Heller, and C Langebartels. 1998. Ozone: An abiotic elicitor of plant defence reactions. *Trends in Plant Science* 3: 47-50.

Sant'Anna, S M R, M P Esposito, M Domingos, and S R Souza. 2008. Suitability of *Nicotiana tabacum* 'bel W3' for biomonitoring ozone in São Paulo, Brazil. *Environmental Pollution* 151: 389-94.

Severino, J F, K Stich, and G Soja. 2007. Ozone stress and antioxidant substances in *Trifolium repens* and *Centaurea jacea* leaves. *Environmental Pollution* 146: 707-714.

Sharkey, T D, E L Singsaas, P J Vanderveer, and C Geron. 1996. Field measurements of isoprene emission from trees in response to temperature and light. *Tree Physiology* 16: 649-54.

Sharma, Y K and K R Davis. 1997. The effects of ozone on antioxidant responses in plants. *Free Radical Biology and Medicine* 23: 480-488.

Singh, E, S Tiwari, and M Agrawal. 2010. Variability in antioxidant and metabolite levels, growth and yield of two soybean varieties: An assessment of anticipated yield losses under projected elevation of ozone. *Agriculture, Ecosystems, and Environment* 135: 168-177.

Smirnoff, N and G L Wheeler. 2000. Ascorbic acid in plants: Biosynthesis and function. *Critical Reviews in Plant Sciences* 19: 267-290.

Smirnoff, N. 1996. The function and metabolism of ascorbic acid in plants. *Annals of Botany*, 78:661-669.

Smith, W B, P D Miles, J S Vissage, and S A Pugh. 2003. Forest resources of the United States, 2002. USDA Forest Service, North Central Research Station. *General Technical Report. NC-241*137.

Takahama, U and T Oniki. 1992. Regulation of peroxidase-dependent oxidation of phenolics in the apoplast of spinach leaves by ascorbate. *Plant and Cell Physiology* 33: 379.

Tausz, M, N E Grulke, and G Wieser. 2007. Defense and avoidance of ozone under global change. *Environmental Pollution* 147: 525-31.

Tausz, M, M Gonzalez-Rodriguez, S Wonisch, J Peters, D Grill, D Morales, and M S Jimenez. 2004. Photostress, photoprotection, and water soluble antioxidants in the canopies of five Canarian laurel forest tree species during a diurnal course in the field. *Flora* 199: 110-119.

Then, C, K Herbinger, V C Luis, C Heerdt, R Matyssek, and G Wieser. 2009. Photosynthesis, chloroplast pigments, and antioxidants in *Pinus canariensis* under free-air ozone fumigation. *Environmental Pollution* 157: 392-395.

Treshow, M. 1970. Ozone damage to plants. *Environmental Pollution (1970)* 1: 155 - 161.

Turcsanyi, E, T Lyons, M Plochl, and J Barnes. 2000. Does ascorbate in the mesophyll cell walls form the first line of defence against ozone? Testing the concept using broad bean (*Vicia faba* L.). *Journal of Experimental Botany* 51: 901.

Valpuesta, V and M A Botella. 2004. Biosynthesis of l-ascorbic acid in plants: New pathways for an old antioxidant. *Trends in Plant Science* 9: 573-7.

Wang, S Y and W Zheng. 2001. Effect of plant growth temperature on antioxidant capacity in strawberry. *Jourrnal of Agriculture and Food Chemistry* 49: 4977-4982.

Wellburn, F A M and A R Wellburn. 1996. Variable patterns of antioxidant protection but similar ethene emission differences in several ozone-sensitive and ozone-tolerant plant selections. *Plant Cell and Environment* 19: 754-760.

Wildi, T, and W Lutz. 1996. Antioxidant Composition of Selected High Alpine Plant Species From Different Altitudes *Plant, Cell and Environment,* 19: 138-146.

Yan, K, W Chen, G Y Zhang, S Xu, Z L Liu, X Y He, and L L Wang. 2010. Elevated CO_2 ameliorated oxidative stress induced by elevated O_3 in *Quercus mongolica*. *Acta Physiol Plant* 32: 375-385.

Zhang, S and D F Klessig. 2001. MAPK cascades in plant defense signaling. *Trends in Plant Science* 6: 520-527.

Zheng, Y, T Lyons, J H Ollerenshaw, and J Barnes. 2000. Ascorbate in the leaf apoplast is a factor mediating ozone resistance in *Plantago major*. *Plant Physiology Biochemistry* 38: 403-411.

Table 1: Buffer gradient used for sample runs indicating concentration of acetonitrile [HPLC grade] and 50mM ammonium acetate, pH 6.8, 90:10 v/v (Buffer A) and 70% ACN, 30% ammonium acetate (Buffer B) throughout one trial period (20 min).

Time (min)	Buffer A	Buffer B
0.0	90	10
2.0	90	10
4.0	85	15
6.0	80	20
8.0	80	20
10.0	75	25
12.0	75	25
14.0	70	30
16.0	70	30
18.0	75	25
20.0	80	20

Table 2: Analysis of variance for the effects of block (random effect), native/invasive status (fixed effect), and species (random effect) results on concentration of constitutive levels of ascorbic acid (dependent variable). Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.

Effect	ndf	ddf	*F or* X^2
Block	2	---	---
Status	1	9	6.15*
Species(Status)	9	---	26.93***

Table 3: Repeated measures analysis of variance for the effects of ozone treatment (fixed effect), native/invasive status (fixed effect), time (before or after ozone exposures, fixed effect), species (random effect), ozone treatment* status interaction (fixed effect), ozone treatment*time (fixed effect), and native/invasive status*time (fixed effect) on the concentration of levels of ascorbic acid (dependent variable). Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.

Effect	ndf	ddf	F or X^2
Ozone	2	8	2.96
Status	1	6	1.89
Time	1	54	2.01
Species(Status)	6	---	6.85**
Ozone*Status	2	8	0.20
Ozone*Time	2	54	6.87**
Status*Time	1	54	1.44

Table 4: Analysis of variance for the effects of ozone treatment (fixed effect), native/invasive status (fixed effect), and species (random effect) on the difference in constitutive and post-exposure ascorbic acid concentrations (dependent variable). Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.

Effect	ndf	ddf	F or X^2
Ozone	2	8	6.87**
Status	1	5	0.66
Species(Status)	6	---	0.00
Ozone*Status	2	8	0.43

Table 5: Analysis of variance for the effects of species (random effect), constitutive ascorbic acid levels (fixed effect), and ozone treatment (fixed effect) on the change of net photosynthesis (dependent variable, Elton Chapter 1). Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.

Effect	ndf	ddf	*F or* X^2
Species	9	---	2.04*
ASA	1	37	0.14
Ozone	1	37	14.22***
ASA*Ozone	1	37	0.13

Table 6: Analysis of variance for the effects of ozone treatment (fixed effect), species (random effect), and post-exposure ascorbic acid levels (fixed effect) on the change of net photosynthesis (dependent variable, Elton Chapter 1). Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.

Effect	ndf	ddf	*F or* X^2
Species	9	---	16.79***
ASA	1	7	6.35*
Ozone	1	7	7.07*
ASA*Ozone	1	7	13.88*

Figure Legend:

Figure 1: Hyrdophilic interaction liquid chromotography chromatographs for 20 μl of *Quercus rubra* leaf extract. Ascorbic acid peaks can be seen for a) replicate 1, b) replicate 2, c) replicate 3, and d) replicate 4 including the addition of ascorbic acid standard.

Figure 2: Constitutive ascorbic acid (ASA) means for each species. Species codes are as follows: *A. rubrum* (ARU), *A. altissima* (AAL), *A. platanoides* (APL), *Q. acutissima* (QAC)*, P. serotina* (PSE), *Q. alba* (QAL), *C. glabra* (CGL), *P. tomentosa* (PTO), *L. tulipifera* (LTU), *Q. rubra* (QRU). Native species are indicated by a # following species codes. Error bars represent 95 % confidence limits. Different letters on error bars indicate significant difference at $p<0.05$ tested using Tukey's post hoc comparisons.

Figure 3: Constitutive ascorbic acid (ASA) means for invasive and native species groups. Error bars represent 95 % confidence limits. Different letters on error bars indicate significant difference at $p<0.05$ tested using Tukey's post hoc comparisons.

Figure 4: Ascorbic acid (ASA) levels by ozone exposure treatment for constitutive and post-exposure levels. Different letters on error bars indicate significant difference at $p<0.05$ tested with a-priori contrasts comparing constitutive and post-exposure levels within each of the ozone treatments.

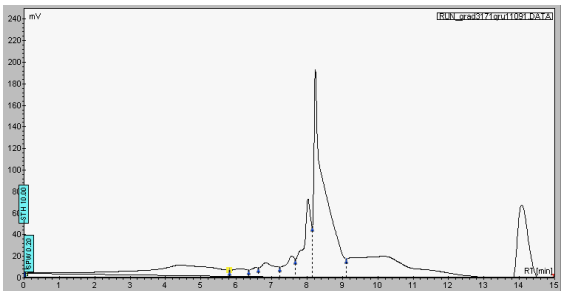
Figure 5: Post-exposure ascorbic acid (ASA) levels of each species. Species codes are as follows: *A. rubrum* (ARU), *A. altissima* (AAL), *A. platanoides* (APL), *Q. acutissima* (QAC)*, P. serotina* (PSE), *Q. alba* (QAL), *C. glabra* (CGL), *P. tomentosa* (PTO), *L. tulipifera* (LTU), *Q. rubra* (QRU). Native species are indicated by a # following species codes. Different letters on error bars indicate significant difference at $p<0.05$ tested using Tukey's post hoc comparisons.

Figure 6: The change of net photosynthesis (an estimate of ozone tolerance) plotted against constitutive ascorbic acid (ASA) levels. The plotted line indicates the line of best fit.

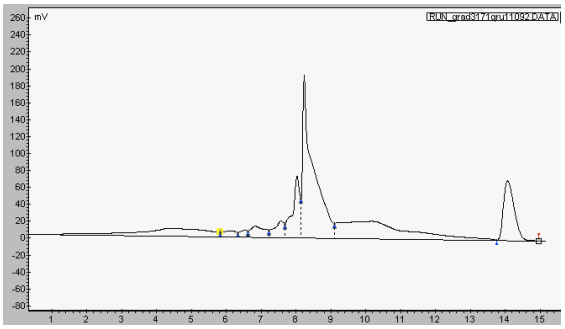
Figure 7: The change of net photosynthesis (an estimate of ozone tolerance) plotted against post-exposure acorbic acid (ASA) levels for medium and high ozone treatments. The plotted line indicates the line of best fit.

Figure 8: Constitutive and post-exposure ascorbic acid (ASA) for each species. Species codes are as follows: *A. rubrum* (ARU), *A. altissima* (AAL), *A. platanoides* (APL), *Q. acutissima* (QAC)*, P. serotina* (PSE), *Q. alba* (QAL), *C. glabra* (CGL), *P. tomentosa* (PTO), *L. tulipifera* (LTU), *Q. rubra* (QRU). Native species are indicated by a # following species codes. Error bars indicate 95 % confidence intervals.

Figure 1a:



b:



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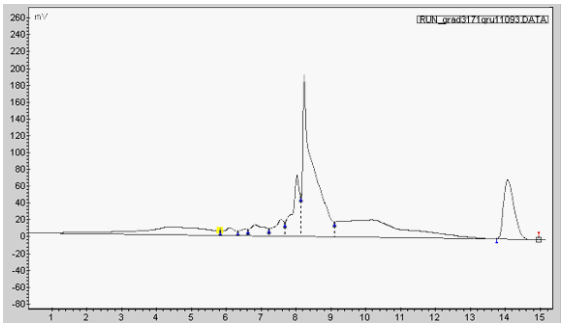

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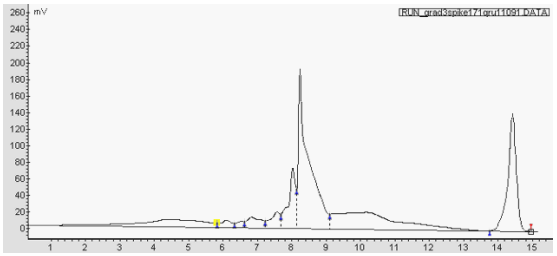

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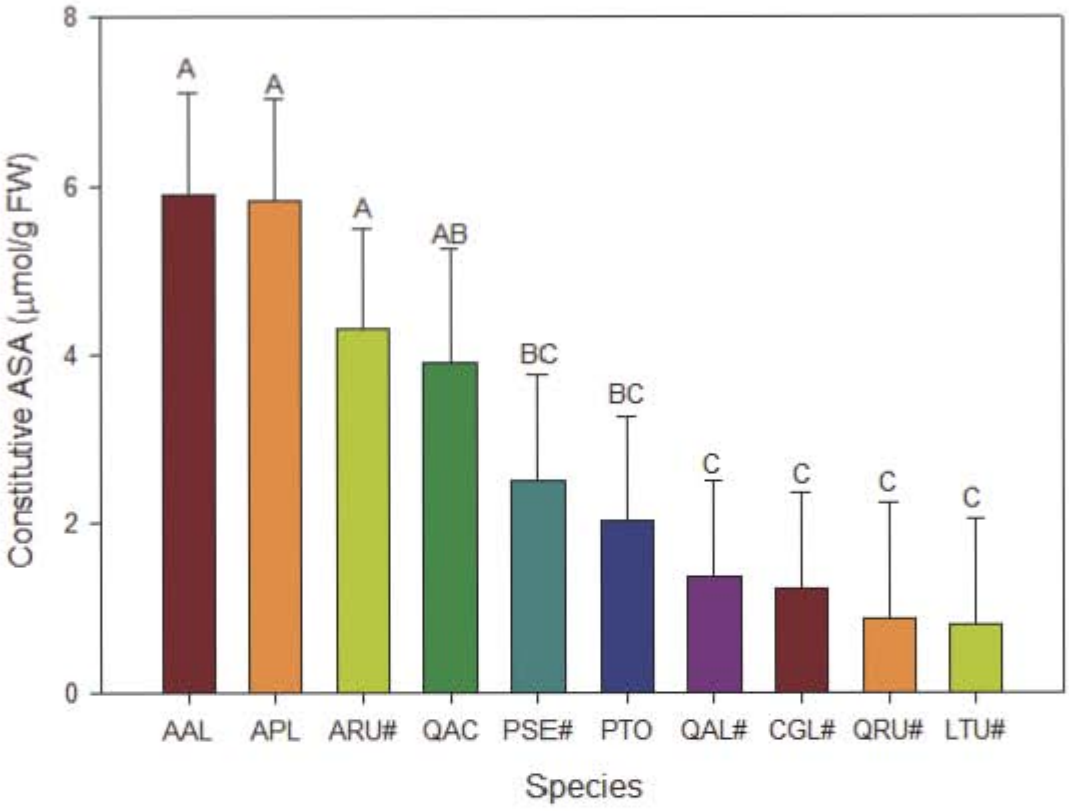

Constitutive ASA (µmol/g FW)
8
6
4
2
0
A
A
A
AB
BC
BC
C
C
C
C
AAL
APL
ARU#
QAC
PSE#
PTO
QAL#
CGL#
QRU#
LTU#
Species

Figure 3:

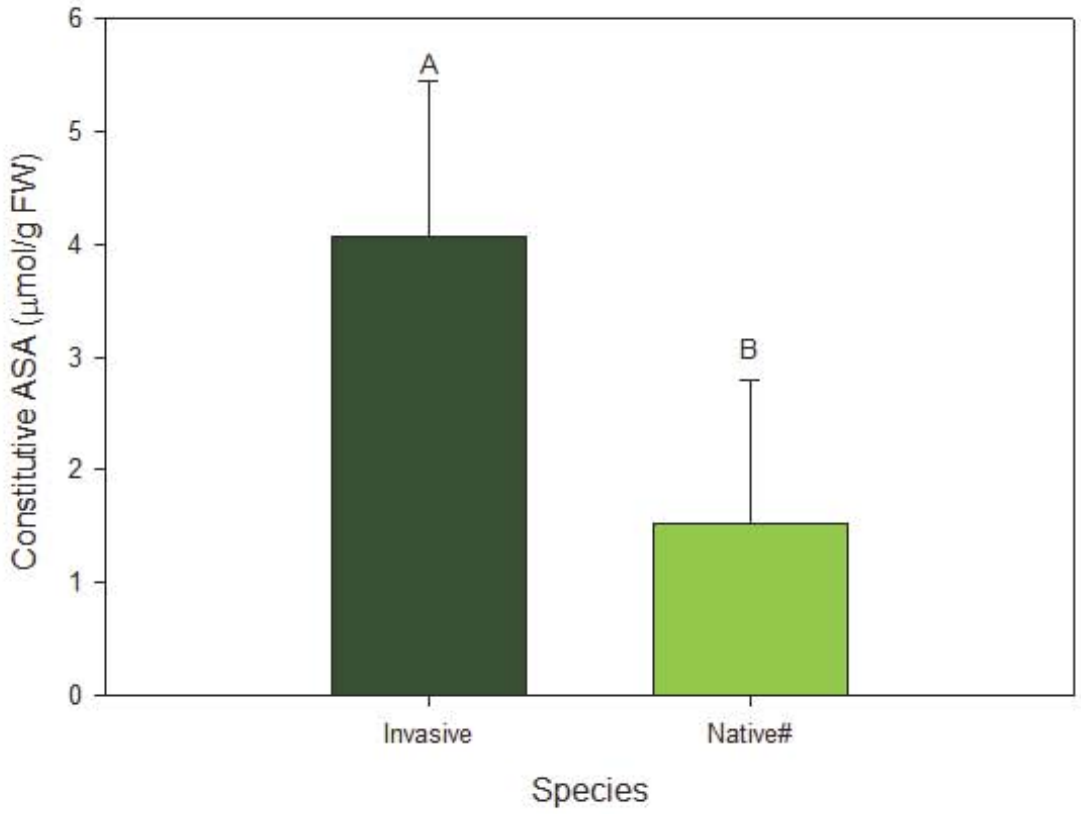

6
5
4
3
2
1
0
Constitutive ASA (µmol/g FW)
A
B
Invasive
Native#
Species

Figure 4:

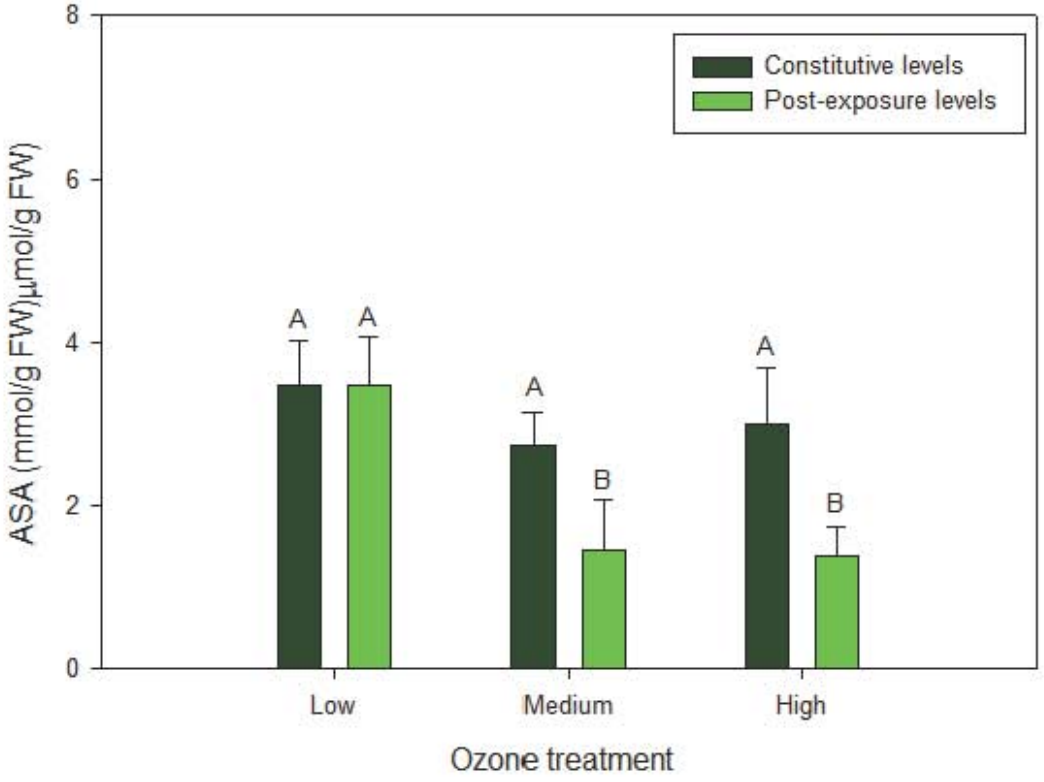

8
6
4
2
0
ASA (mmol/g FW)µmol/g FW)
Constitutive levels
Post-exposure levels
A
A
A
B
A
B
Low
Medium
High
Ozone treatment

Figure 5:

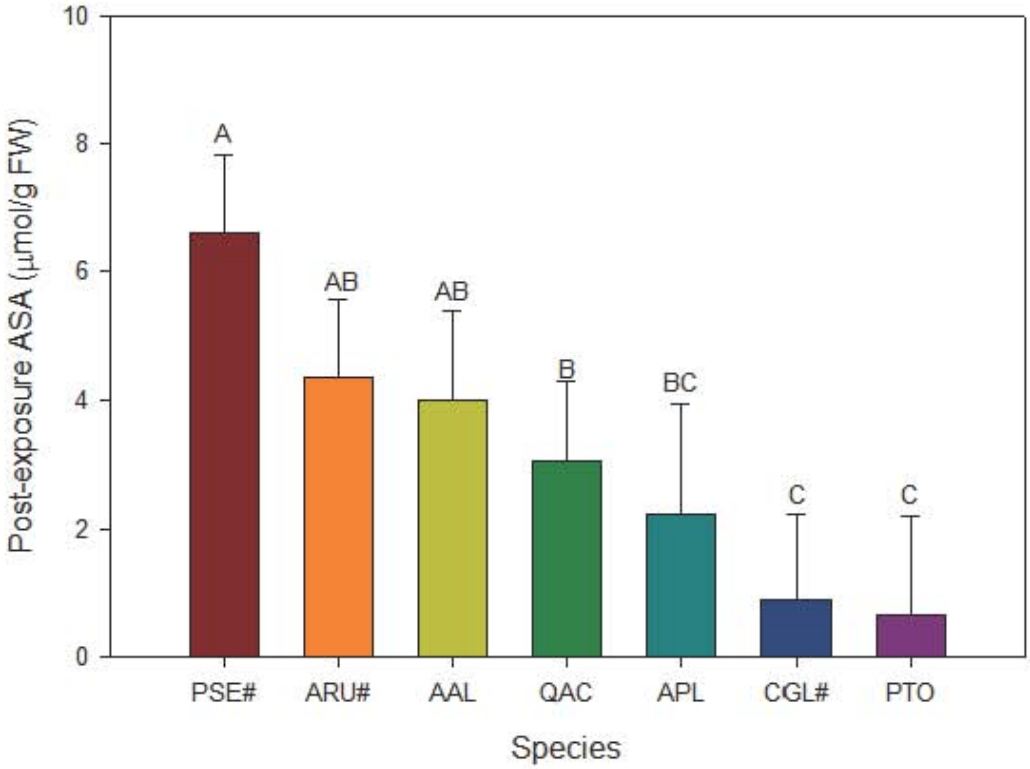

10
8
6
4
2
0
Post-exposure ASA (µmol/g FW)
A
AB
AB
B
BC
C
C
PSE#
ARU#
AAL
QAC
APL
CGL#
PTO
Species

Figure 6:

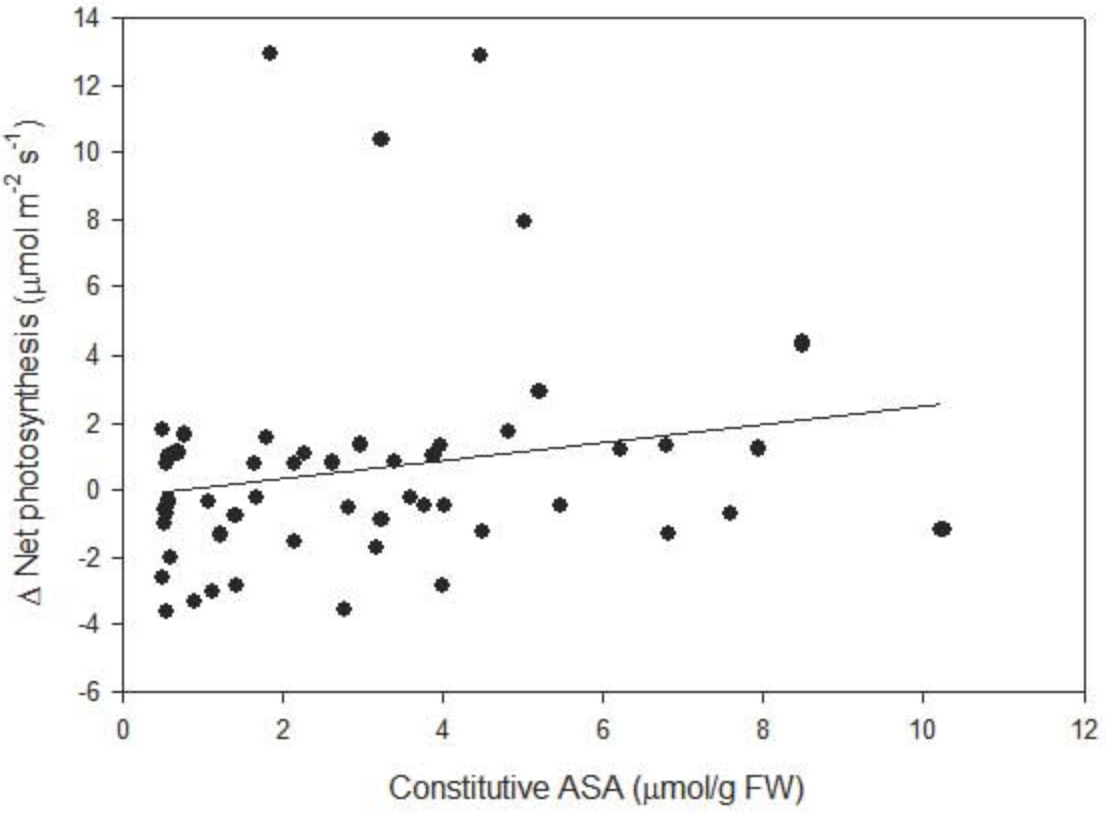

Δ Net photosynthesis (µmol m-2 s-1)
Constitutive ASA (µmol/g FW)
14
12
10
8
6
4
2
0
-2
-4
-6
0
2
4
6
8
10
12

Figure 7:

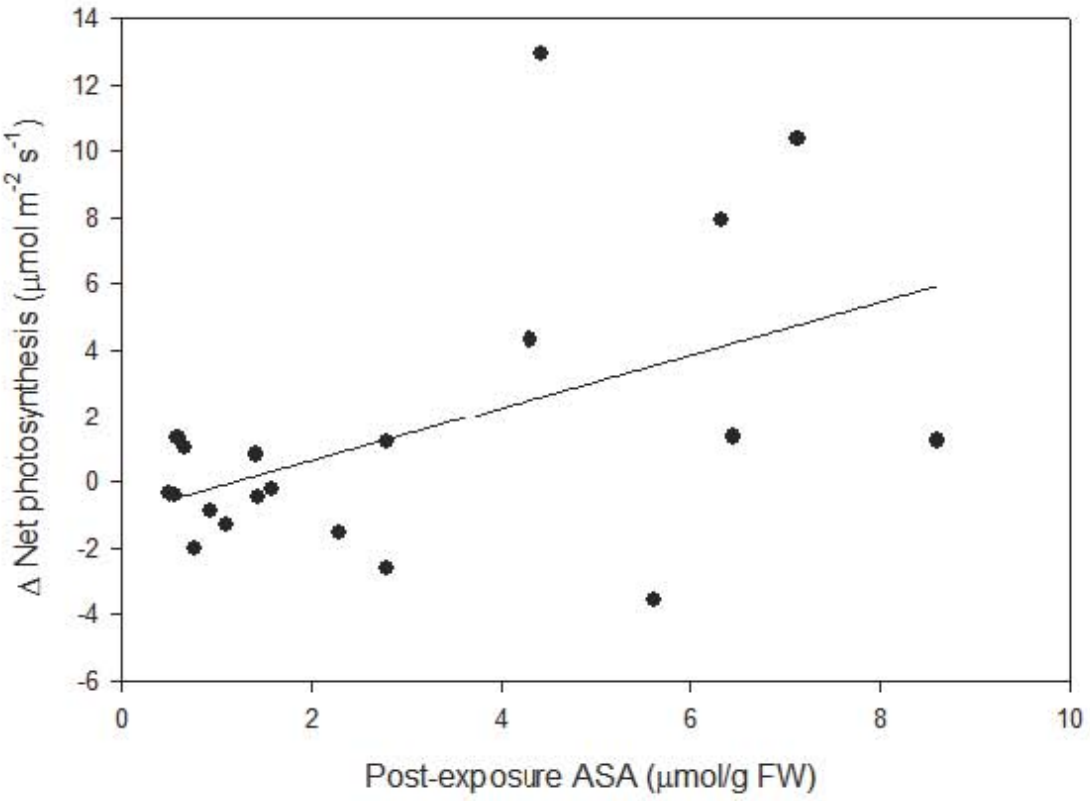
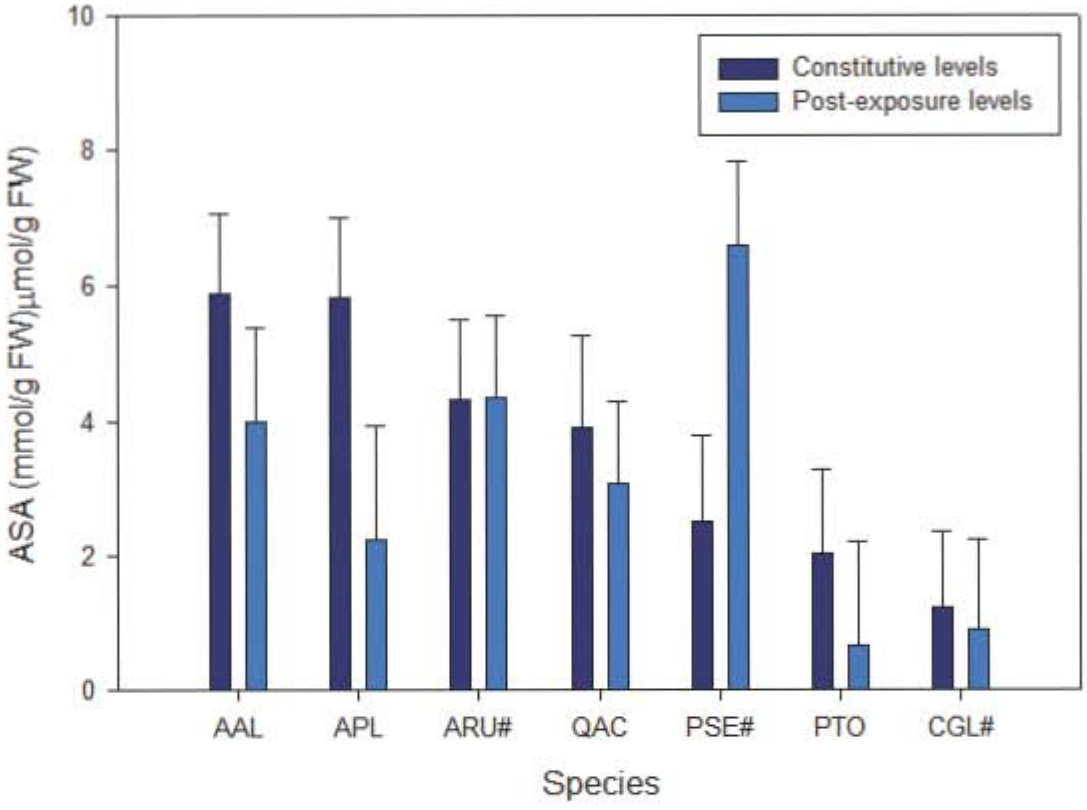

14
12
10
8
6
4
2
0
-2
-4
-6
0
2
4
6
8
10
Δ Net photosynthesis (μmol m-2 s-1)
Post-exposure ASA (μmol/g FW)

Figure 8:


Constitutive levels
Post-exposure levels
ASA (mmol/g FW)µmol/g FW)
10
8
6
4
2
0
AAL
APL
ARU#
QAC
PSE#
PTO
CGL#
Species

CHAPTER 3
Forecasting the effect of ozone on mixed deciduous forests

Abstract

Ozone pollution can cause extensive damage to trees, but species vary significantly in their tolerance to ozone exposure. This variation could create a restructuring role for ozone in forests that are experiencing high pollution stress. To assess the successional dynamics of a forest community exposed to different ozone regimes, an individual physiological based model (TREGRO) was linked to a spatially explicit forest stand gap model (ZELIG). Individual mature trees were modeled for three years in TREGRO for 13 broadleaf deciduous tree species including native and invasive species commonly found in the Mid-Atlantic region of the United States. The results from the TREGRO model were used to modify the growth rate for the response of each species to ozone within the ZELIG model. The ZELIG trials modeled the succession of a forest on a recently abandoned field in a low, medium, and high ozone environment for 200 years. One hundred independent simulations were run for each ozone regime. High ozone stress facilitated high species diversity by weakening the competitive ability of ozone sensitive species, which typically exhibited dominance. Ozone sensitivity reduced the abundance of some species (both dominant and non-dominant species) while ozone and shade tolerance enabled other species to increase in abundance. Though new species failed to enter the canopy with increasing ozone stress, tree size decreased and changes in canopy dominance occurred due to varying ozone tolerance among species. High ozone stress caused a thinning of trees during early forest growth, which enhanced the growth of the remaining trees. In contrast, moderate ozone levels did not lead to tree thinning, but impaired tree growth due to the positive interaction between increased competition

and ozone stress. Ozone is one of many stressors that currently affects Mid-Atlantic forest communities and is likely to play an important role in the reorganization of forest structure and species composition.

Introduction

Forest communities are exposed to a myriad of disturbances and stressors. Though forest systems are often resilient to various disturbance regimes, novel anthropogenically driven stressors can cause alterations in forest community dynamics (Foster et al. 1997). One such stressor, ground-level ozone (O_3), has increased in concentration over the last 100 years and is predicted to remain a problem for forest communities during the next century (Fowler et al. 1999, Vingarzan 2004, Dentener et al. 2006, Fowler et al. 2008). Little information is available on the effects of O_3 on forest communities as most studies examine the direct effects of O_3 on a single or small number of commercially important tree species (e.g., Long and Davis 1991, Samuelson and Edwards 1993, He et al. 2006, Prozherina et al. 2009). This study focuses on the effects of ground-level O_3 pollution on the diverse Mid-Atlantic forest community, especially the opportunity this stress may provide for invasive species.

Forests span nearly one-third of all land worldwide, and provide habitat for two-thirds of terrestrial species (FAO 2006). Forest ecosystems provide essential environmental services including soil production, erosion control, watershed protection, climate control, oxygen production, and carbon storage. In addition to supporting the biosphere, forests also support many economies by providing renewable resources

including timber and more than 5000 other products of economic importance (Patton 1992). Declines and removal of this highly valued system can have significant detrimental impacts on humans. In extreme cases, loss of forests can lead to the elimination of entire populations such as those once inhabiting Easter Island or the Nazcan people of Peru (Rolett and Diamond 2004, Beresford-Jones et al. 2009). Deforestation occurred at a rate of 5.2 million hectares per year between 2000 and 2010 (FAO 2010) leaving only one-third of all forests as primary forests (Smith et al. 2003). All of these factors highlight the need for better stewardship of forested land and the prevention of further degradation.

The diversity of native plants is further threatened by invasive species (Vitousek 1990, Ehrenfeld 1997, With 2002, Seabloom 2003, Lake and Leishman 2004). Invasive species are non-native species introduced to an area where they successfully establish and cause both economic and environmental damage. Ecosystems can be negatively affected by invasive species due to alterations in diversity (Woods 1993, Wyckoff and Webb 1996, Martin 1999), nutrient dynamics (Vitousek et al. 1987, Holmes and Cowling 1997, Ehrenfeld 2003, Ashton et al. 2005), hydrology (Loope et al. 1988, Melgoza et al. 1990, D'Antonio and Mahall 1991, Gerlach 2000), and disturbance regimes (D'Antonio and Vitousek 1992, D'Antonio 2000, Tunison et al. 2001). In forest systems, invasive tree species have caused reduced diversity of native species. For example, the invasion of *Acer platanoides* near Ithaca, New York caused reductions in the richness of young native trees due to increased competition for light (Martin 1999). Other invasive tree species (e.g., *Ailanthus altissima*) alter the soil chemistry of a forest, creating an

inhospitable environment for native species (Heisey 1990, Lawrence et al. 1991, Gomez-Aparicio 2008). Invasive species may also alter the successional dynamics of a forest causing long-term changes in the structure and function of the system.

The long-term influence of an invasive tree species depends on whether it is ephemeral or persistent within a forest community. Unlike invasive species of other growth forms, invasive trees rarely dominate forest systems (Forman and Elfstrom 1975, Baker 1986, Robertson et al. 1994). Moreover, closed canopy forests are considered highly resistant to invasive species (Fox and Fox 1986, Naeem et al. 2000, Sakai et al. 2001) though exceptions have been noted (e.g., Ehrenfeld 1997). Prior to the closure of the forest canopy, invasive tree species are able to reach greater abundances than native species leaving a more enduring impact on the community (Kay 1994, Robertson et al. 1994, Mazia et al. 2001). The presence of a transient invasive species may be regarded as a preliminary step toward the recovery of a forest during secondary succession, and not viewed as a major threat to the species composition of the climax forest. However, if a forest is exposed to repeated stress events (e.g., elevated ground-level O_3) that create small canopy gaps or premature senescence, invasive species may be able to persist and alter forest diversity patterns and structure. It is likely that invasive species influence recently disturbed areas to a greater extent than mature forests because recolonization inhibits the growth of native species and slows the successional dynamics of a given forest system (Marcano-Vega et al. 2002, Lichstein et al. 2004). In this case, the persistence of invasive species may also be aided if stress events favor their growth.

Ground-level O_3 concentrations have increased significantly since the Industrial Revolution due to the increased production of nitrogen oxides and volatile organic compounds. Background levels of O_3 before this time were close to 10 ppb (Marenco et al. 1994) but now are found greater than 40 ppb in the northeastern United States. Hourly O_3 levels above 60 ppb (AOT60), the threshold considered harmful for trees, are currently experienced by one-fourth of all forests (Fowler et al. 1999). By 2100, the area of forests affected by elevated O_3 concentrations is predicted to increase two-fold, with forests in the Northern Hemisphere being the most negatively impacted (Karnosky et al. 2005). Elevated ground-level O_3 can impair photosynthesis (Grams et al. 1999, Wittig et al. 2009, Elton Chapter 1), stomatal conductance (Wittig et al. 2007, Elton Chapter 1), growth rates (Bartholomay et al. 1997, Somers et al. 1998, Dittmar et al. 2003, Novak et al. 2007), and biomass accumulation (Karnosky et al 2003, Grams et al. 2007, Karnosky et al. 2007).

Exposure to periodic stress events can ultimately favor stress tolerant species (Dunson et al. 1991, Vinebrooke et al. 2004, McDowell et al. 2008, Tylianakis et al. 2008, Angilletta 2009). In particular, chronic and acute O_3 exposure elicits species-specific responses (Kozlowski et al. 1991, Bassow and Bazzaz 1997, Lambers et al. 1998, Vinebrooke et al. 2004, Orendovici-Best et al. 2008, Bassin et al. 2009, Girgzdiene et al. 2009, Luedemann et al. 2009, Baek and Woo 2010, Elton Chapter 1). In Lithuania, ground-level O_3 levels have increased by 0.4 ppb each year since 1982, and already the growth rates and abundances of sensitive species (*Fraxinus excelsior*, *Betula* spp., *Alnus icana*) have decreased throughout the O_3 affected area (Giergzdiene et al. 2009). Ozone

sensitivity is greatest among broadleaf tree species (Matyssek et al. 2010), which emphasizes the need to study systems such as Mid-Atlantic hardwood forests.

Stress events benefit stress tolerant species, and invasive species often perform better in chronically stressed areas (Hobbs 1989, Mack and D'Antonio 1998, Naeem et al. 2000, Mazia et al. 2001). Though invasive species, as a group, did not differ from native species in response to O_3 in exposure chamber experiments, strong species-specific responses in gas-exchange occurred (Elton Chapter 1) that might favor particular invasive species. One invasive species of particular concern is *Quercus acutissima*, a shade tolerant pioneer species, which was found to have a higher growth rate in polluted conditions compared to clean air. If native species incur major reductions in growth rates after O_3 exposures (e.g., *Liriodendron tulipifera*, *Prunus serotina*) and experience a loss of competitive ability, forests may be dramatically restructured by the presence of species like *Q. acutissima*. Therefore, it is important to scale-up O_3 responses from chamber experiments and allow for competition among species to predict how species assemblages may respond to changes in O_3 concentrations.

To evaluate the influence of stressors on ecosystems, one must not be bound by the spatial and temporal limits of experiments. Forest systems have temporal dynamics that are far too slow to permit the evaluation of changes in physiological processes during the lifetime of a tree (50-350 years). The evaluation of changes in biodiversity over a successional time scale (150-1000 years) is even more challenging. One solution to the temporal limitations of studying forest systems is to use models that integrate short-term data over long time scales (Laurence and Anderson 2003). These tree-based models can

be manipulated in order to assess the impact of various stresses on physiological processes (Newham 1964, Botkin et al. 1972, Ek and Monserud 1974, Shugart 1984, Smith and Urban 1988, Friend et al. 1993, Laurence et al. 1993, Pacala and Hurtt 1993, Weinstein and Yanai 1994, Busing 1995, Ollinger et al. 1998).

Gap models are individual-based models that include physiological processes to allow the estimation of forest succession on landscape scales. To evaluate the response of forest systems, gap models use a series of algorithms to model the establishment, diameter growth, and mortality of each tree in a given area, similar to individual tree growth models. These processes are often modified by the interaction of climate, stand competition, and variability of site conditions. Physiological and demographic species parameters (e.g., shade tolerance, drought tolerance, nutrient requirements, tree shape, maximum age) allow gap models to create a dynamic prediction of species interactions. Because gap models process information for each individual tree and allow asymmetric competition, they are very useful when evaluating responses that vary on the species level. The response of each species is estimated by calculating an optimal growth response, and deviations of suboptimal conditions are represented by various scalars (e.g., abiotic and biotic influences). Inter-tree competition for light is modeled using the Beer-Lambert law, and functions of tree height and leaf area index determine light extinction within the canopy. Competition for water and soil nutrients is often modeled using a linear decrease in growth as stand basal area increases.

Shugart and West developed one of the first gap models, FORET (FORests of Eastern Tennessee), which modeled 33 species common to southern Appalachian

deciduous forests (1977). FORET was derived from the JABOWA model (Botkin et al. 1972) that simplified many of the complex algorithms of previous tree models (Lee 1967, Mitchell 1969, Bella 1971, Munro 1974) to create a more broadly applicable platform. FORET was validated for pre- and post-chestnut blight forest compositions (Shugart and West 1977) and was used to reconstruct forests of eastern Tennessee based on prehistoric pollen records (Solomon et al. 1980, Solomon et al. 1981, Solomon and Webb 1985) and periglacial records (Solomon and Shugart 1983, Shugart 1984).

Multiple models were derived from the FORET framework, and continue to be used to model Mid-Atlantic forests. For example, the FORENA (FORests of Eastern North America) model (Solomon 1986) was one of the first to show that climate change might result in major diebacks in Mid-Atlantic forests. This was due to the fact that climatic conditions became inhospitable to extant species at a rate that exceeded tree migration rates.

While prior use of gap models focused on regional spatial scales, the OVALIS model (Harrison and Shugart 1990) was designed to examine smaller spatial scales, in particular the Shaver Hollow watershed in Shenandoah National Park. This model used FORET as a framework and included temperature and soil moisture data specific to Shaver Hollow. Parameters were added to classify drought tolerance for each tree species. With this new addition, the OVALIS model accurately predicted (i.e., within 99% confidence intervals) the distribution of dominant forest species in this region (Harrison and Shugart 1990) and showed that gap models could accurately predict forest dynamics for smaller spatial scales when soil moisture and temperature were considered.

Another model developed and tested in the Mid-Atlantic region is the spatially explicit ZELIG model (Smith and Urban 1988, Urban 1990). ZELIG is based on the framework of the FORET model but differs in the spatially explicit nature of the grid space used to simulate a given plot (Urban 1990, Urban et al. 1991). ZELIG was developed to allow nearest-neighbor interactions among grids, including shading of neighboring trees and competition for nutrients. ZELIG incorporated tree canopy shape (crown width, crown length, and crown form), and leaf area portioned throughout the canopy of the tree into the calculation of insolation, using species-specific allometric equations. Thus, ZELIG determined detailed and complex responses to forest gap size, a property that FORET was unable to reproduce (Urban et al. 1991, Weishampel and Urban 1996). The ZELIG model has been used to determine the response of forest systems to climatic changes (Urban et al. 1989, Cumming and Burton 1996, Larocque et al. 2006), disturbances (Smith and Huston 1989, Pabst et al. 2008), and stress conditions (Laurence et al. 2001, Weinstein et al. 2001).

Individual physiological tree growth models effectively scale-up photosynthetic measurements in response to a stressor for an individual leaf. Combining gap models with individual tree growth models allows for the effective assessment of the impact of stress events on forest communities by incorporating interactions of inter-tree competition. Laurence et al. (2001) identified this need to link individual physiological tree growth models with complex stand models and tested the effects of O_3 and precipitation on *Liriodendron tulipifera* and *Pinus taeda* growth using TREGRO (Weinstein and Beloin 1990), an individual tree growth model, and ZELIG (Smith and

Urban 1988), a spatial gap model. Weinstein and Beloin (1990) found that ZELIG predicted much greater effects of O_3 than TREGRO for *P. taeda*. The stress response of an isolated tree may be magnified or reduced when that tree is grown within a forest community due to competition. TREGRO allows for reductions in tree growth to be buffered by stored resources, whereas ZELIG quickly disadvantages trees with reduced growth rates by way of neighboring tree competition for light. The incorporation of the species-specific physiological responses measured in TREGRO within the ZELIG model predicted a more accurate composition of *L. tulipifera* (Weinstein et al. 2001) and *Pinus ponderosa* (Weinstein et al. 2005), which supports the linking of the two models. The studies that have used TREGRO and ZELIG have acquired O_3 response data for only one to two species, but using O_3 response data for a large number of species could strengthen this approach.

In this study, I modeled individual trees of 13 different species using TREGRO to estimate the effect of O_3 on tree growth for three years. Eight native and five invasive tree species that varied in competitive ability and response to elevated ground-level O_3 were included. The results from TREGRO modified the growth of the species within the ZELIG model to simulate forests of varying O_3 regimes. The goals of this study were to evaluate: 1) the effect of O_3 on forest species composition, and 2) the effect of O_3 on forest structure and tree size. Differences in forest composition across an O_3 gradient would be suggestive of a structuring role of O_3. Species composition and forest structure were predicted to follow trends of species O_3 sensitivity noted by Elton (Chapter 1).

Methodology

TREGRO

Model description

The TREGRO model (Weinstein and Beloin 1990, Weinstein et al. 1991, Laurence et al. 1993, Weinstein and Yanai 1994, Weinstein et al. 2005) is an individual and physiological process based model designed to simulate the growth of a tree as it interacts with local climate, soil factors, and ground-level O_3. The parameters and equations used within the model have been validated and verified by a number of studies (Laurence et al. 1993, Retzlaff et al. 2000, Tingey et al. 2001). The model divides the tree into five biomass compartments (fine roots, coarse roots, stem, branches, leaves) and tracks three carbon pools (living structure, dead structure, total nonstructural carbohydrates) within each compartment. The movement of carbon between the biomass compartments is tracked daily, accounting for losses from respiration and senescence. Photosynthesis is modeled hourly for the entire tree based on environmental conditions including O_3 concentrations. Priorities for growth are established by the phenology of each tree species.

Model parameterization

Net photosynthesis rates collected two days after O_3 exposure trials (Elton Chapter 1) for 13 species (native: *Acer rubrum*, *Carya glabra*, *Celtis occidentalis*, *L. tulipifera*, *Prunus serotina*, *Quercus alba*, *Q. rubra*, *Robinia pseudoacacia*, invasive: *Acer platanoides*, *Ailanthus altisimma*, *Paulownia tomentosa*, *Pyrus calleryana*, *Q.*

acutissima) were used to calibrate the TREGRO growth rates. These data were used instead of net photosynthesis values measured during O_3 exposure trials because physiological damage showed a time lag up to 48 hours in some trees after exposures. This group includes early- (*R. pseudoacacia*, *P. serotina*, *A. rubrum*, *L. tulipifera*, *A. altissima*, *P. tomentosa*, *Q. acutissima*), mid- (*A. platanoides*, *A. rubrum*, *C. occidentalis*, *P. calleryana*) and late-successional (*C. glabra*, *Q. alba*, *Q. rubra*) species. Elton (Chapter 1) determined that *L. tulipifera*, *R. pseudoacacia*, *and A. altissima* had the highest growth rates in a low O_3 environment, but after exposure to high O_3 levels the top three performing species were *P. calleryana*, *Q. acutissima*, and *R. pseudoacacia* (Table 1). The O_3 effects on growth were modeled using decreases in maximum carboxylation rate controlled by three factors: 1) threshold O_3 (80 ppb), 2) threshold cumulative O_3 uptake, below which no O_3 effect takes place, and 3) a slope of reduction of maximum carboxylation rate as cumulative O_3 uptake increases (Weinstein et al. 1991, Rubin et al. 1996). The O_3 threshold of 80 ppb was chosen due to the decreases in photosynthesis that occur in many plants at this level (Lefohn and Foley 1992, Krupa et al. 1998, Musselman et al. 2006, Elton Chapter 1).

Hourly temperature and precipitation data for 2007-2009 were collected from a weather station located at the University of Virginia's Blandy Experimental Farm. Ozone data were taken from the nearest O_3 monitoring station in Winchester, Virginia, which is maintained by the Department of Environmental Quality (DEQ). Ozone exposure regimes were manipulated to investigate the response of an individual tree to the stress of ground-level O_3. The control simulation was analogous to scrubbed air (0

ppb), and was used as the baseline of the model in order to calculate an estimate of O_3 sensitivity growth reductions (Eq. 1). The other O_3 regimes included low (ambient, 0-76 ppb), medium (1.5x ambient, 0-114 ppb), and high O_3 (3.0x ambient, 0-228 ppb). The ambient O_3 conditions were taken from the DEQ O_3 data set, and any value above 80 ppb was reduced to 65 ppb in order to model a low O_3 environment.

The TREGRO model required additional information to set tree physiology parameters. Biomass values for mature trees were compiled from numerous sources (Appendix I) for each species. The program followed the assumption that soil water potential less than -100 J/kg will cause a linear decrease in the photosynthetic rate, which will stop altogether after water potential reaches -1500 J/kg (Weinstein et al. 2005).

Model runs

Three-year simulations were performed for each species. Equation 1 was used to standardize O_3 stress relative to control biomass. This standardized stress (Y) was used to modify the growth rate parameters for each species in ZELIG (Laurence et al. 2001):

$$Y = \frac{FinalBiomass_{Ozone} - FinalBiomass_{Control}}{FinalBiomass_{Control}} \quad (1)$$

This equation expresses the final biomass for O_3 treatment relative to the biomass for control treatment. Therefore, a value of Y less than zero indicates the degree of O_3

sensitivity. A value of Y equal to zero represents O_3 tolerance, and a value of Y greater than zero represents improved growth under O_3 stress. The sum of one and Y was calculated, and this value was then multiplied by the growth rates determined at the low O_3 trials (serving as the control) and used as growth rate parameters for ZELIG.

ZELIG

Model description

ZELIG is a spatially explicit forest stand gap model (Smith and Urban 1988, Urban et al. 1993) based on the foundation of the JABOWA (Botkin et al. 1972) and FORET (Shugart and West 1977) models. ZELIG allows for the incorporation of spatial and light geometry effects (Larocque et al. 2006), which are known to affect O_3 damage in a forest community. The ZELIG model has been widely applied to forest ecosystems in North America (Laurence et al. 2001, Larocque et al. 2006, Larocque et al. 2007, Larocque et al. 2010). ZELIG simulates competition among species in 25 400-m^2 plots by modeling the life cycle of each tree in response to environmental conditions. Tree growth, mortality, and regeneration are the major factors that determine stand dynamics. Tree growth and establishment are modeled based on maximum potential behavior, and then that behavior is constrained due to limiting light, soil moisture, soil nutrients, and ambient temperature. Mortality is modeled by two functions: a density-dependent competition/suppression function and an ambient density-independent function. I incorporated species-specific parameters (e.g., growing degree day minimum and

maximum, drought tolerance, shade tolerance, maximum age) to allow for robust predictions of forest dynamics on successional temporal scales (Larocque 2008).

Model parameterization

All trials were started from bare ground and stocking potential was the same among all species. Soil field capacity and wilting point in the ZELIG model were parameterized using soil texture information from Blandy Experimental Farm (Wang et al. 2010). The temperature and precipitation 30-year monthly means and standard deviations were calculated from the National Climatic Data Center (NCDC) database for Winchester, Virginia. The ZELIG model used these climatic data to stochastically generate the precipitation and temperature of each month throughout the simulated growing season.

Model runs

Each O_3 regime was assigned to a 1 ha block in a single simulation. Each block experienced the same weather conditions throughout a given simulation. One hundred simulations were run and randomly varied in weather conditions. The simulations ran for a total of 200 years in order to evaluate the community response to O_3 stress in a recently abandoned field. Preliminary analyses demonstrated that means for species abundance, diameter at breast height (DBH), and height stabilized well before 100 simulations, indicating that the estimates reported are near the parametric means for the simulated O_3 conditions.

Model validation

Model validation is important to test the strength of a model and its ability to simulate forest systems. However, the species included in this study represent only a small subset of the pool of potential interacting species in an existing Mid-Atlantic forest, making validation problematic. The forest stands predicted by this model therefore may not match any particular forest. In this study, model validation will be regarded as successful if the species composition and structure of the modeled forest are comparable to those known to occur in the Mid-Atlantic region. This was tested by running ZELIG without invasive species in a low O_3 environment.

Statistical analyses

To test for differences in forest composition and structure, a multivariate analysis of variance was run on data from 50, 100, 150, and 200 years of simulation. To test for the effect of O_3 on forest composition, the relative abundance of each species was calculated for each plot. These relative abundances served as the dependent vector in a two-way MANOVA with block and O_3 regimes as independent variables (PROC GLM, SAS version 9.1.3; SAS Institute, Cary, NC, USA). When significant O_3 effects were detected, pairwise comparisons of O_3 levels were made with separate MANOVAs. Alpha levels were adjusted with the Dunn-Ŝidák method to correct for multiple comparisons.

The mean DBH and mean height for each species were calculated in each plot. These means served as the dependent vectors for two MANOVAs to evaluate the effect of O_3 on forest structure. Block and O_3 regime served as the independent variables.

Pairwise comparisons of O_3 regimes were again made using separate MANOVAs with the Dunn-Ŝidák correction for multiple comparisons.

To test for differences among O_3 simulations in the relative abundance, DBH, and height of individual species of trees, the overall means for each plot were analyzed with a univariate analysis of variance. This PROC GLM (SAS) analysis was used for each forest age of interest (50, 100, 150, 200 years).

To test for the effects of O_3 regime on species diversity (Shannon index), an ANOVA was run in SAS using PROC GLM for each 50-year interval. The independent variables included O_3 regime (fixed effect) and block (random effects).

In order to evaluate similarity in community composition across O_3 simulations, I calculated pairwise Spearman rank correlations using species mean abundance. Tests were performed at each 50-year time step.

Results

Model validation

Results from the model validation simulations demonstrated the dominance of shade intolerant early-successional species (*Robinia pseudoacacia*, *Liriodendron tulipifera*, *Prunus serotina*) within the first 50 years of the simulation (Fig. 1). After canopy closure (~100 years), the shade tolerant mid-successional *Acer rubrum* became the dominant species. It was not until after canopy closure that the late-successional hickory and oak species began to increase in number (Fig. 1). Pignut hickory gained dominance after 150 years and remained the dominant species until the end of the

simulation (year 200) (Fig 1). This contrasts with the typical *Quercus* spp. dominance found throughout the Mid-Atlantic region (Braun 1950, Day 1953, Buell et al. 1954, Buell 1957, Little 1974, Christensen 1977, Russell 1980, Stephenson 1986, Nowacki and Abrams 1991, Seischab and Orwig 1991, Abrams and Nowacki 1992). However, the model simulation did not incorporate disturbance patterns (e.g., fire) known to support the dominance of *Quercus* spp. (Abrams 1992). Furthermore, mature forests in the Northern Piedmont and in Giles County, Virginia have similar species compositions as those modeled by the ZELIG simulations (Table 2). The tree density of the model stand (3500-5000 stems ha^{-1}) was similar to the density of forests measured in the Mid-Atlantic region (3000-5500 stems ha^{-1}) (Braun 1950, Christensen 1977, Stephenson 1986, Nowacki and Abrams 1991).

Forest species composition

In the early stages of forest growth (0-50 years), all 13 species were present in the low O_3 forest. At this time, the forest had a majority of fast growing pioneer species (*Ailanthus altissima*, *P. serotina*, *Q. acutissima*). The fast-growing but short-lived *P. calleryana* was the least abundant species (Fig. 2) indicating that other traits (e.g., seed production) contributed to species abundance. The closing of the forest canopy (~80 years) initiated increases of the shade tolerant *Q. acutissma*, *A. rubrum*, and *C. glabra* and restricted the abundances of the shade intolerant *L. tulipifera* and *R. pseudoacacia* (Fig. 2). By 150 years, the mid-successional *A. rubrum* and late-successional *C. glabra* increased in abundance due to the favoring of shade tolerant species after canopy closure.

In the last 50 years, the shade tolerant understory species, *Q. acutissima* increased in abundance while growth in the understory was reinitiated by the death of canopy trees (Fig. 2). The high relative abundance of *Q. acutissima* (30%) in the understory and *A. altissima* (24%) in the canopy led to the lowest diversity ($F_{3,99}$=12.21, p<0.0001, 1.30 Shannon-Wiener diversity index) and evenness (0.94, Pielou index) among all simulations (Fig. 5). At 200 years, the relative abundance of invasive species (59%) was higher than the native species relative abundance (Fig. 2).

In the medium simulations, O_3 levels were raised by a factor of 1.5 above the low simulation. During early stages of forest growth, the medium O_3 forest was dominated by *P. serotina,* which was 8% more abundant than in the low O_3 forest. *Ailanthus altissima* and *Q. acutissima* ranked as the second and third most abundant species during this time, respectively (Fig. 3). After 100 years, *A. altissima* surpassed *P. serotina* as the most abundant species. Major reductions in *Q. acutissima* (21%) and *C. glabra* (7%) occurred due to their O_3 sensitivity. Increases in relative abundance occurred for the O_3 and shade tolerant *P. serotina* (13%), *A. rubrum* (5%), and *C. occidentalis* (6%). After 150 years, the relative abundances of species were similar to the low O_3 forest except for the greatly decreased abundance of the O_3 sensitive *Q. acutissima*, and the increase in abundance of the O_3 and shade tolerant *C. occidentalis*. Species diversity (1.86) and evenness (0.82) were greater than the low O_3 simulation (Fig. 5). Surprisingly, the medium simulation was the only trial that maintained higher abundances of native species (64%) throughout the entire simulation, possibly pointing to a reduction in competitive ability of invasive species at modeled O_3 levels.

Species composition in the high O_3 forest was similar to the low O_3 forest throughout the simulation, but differed the most at 200 years. As in the low O_3 forest, the high O_3 simulation was dominated by the *P. serotina* (18%), *Q. acutissima* (18%), and *A. altissima* (13%) (Fig. 4). There were decreases in *A. altissima* (8%) and increases in *P. serotina* (9%) and *P. tomentosa* (10%) abundances compared to the low O_3 forest due to the O_3 sensitivity of species in the high O_3 stress environment at 200 years (Fig. 4). Among the three O_3 regimes, the high O_3 simulation had the highest species diversity (1.95) and evenness (0.86) (Fig. 5).

To determine the relationship among the relative abundances of species of the three O_3 regimes at 50-year intervals, a Spearman rank correlation test was performed (Table 3). The greatest similarity was found between high and low O_3 forests. Interestingly, the species composition of the medium O_3 forest was the most unique. Forest compositions were most similar early in the simulations. The species composition of the low and high O_3 forests remained significantly similar throughout the entire simulation. The only period that the low and medium O_3 forests had similar compositions was between 100-150 years.

Species size distribution

To determine if O_3 affected the size distribution of species, both diameter at breast-height (DBH) and the height for the simulated trees were measured at 50, 100, 150, and 200 years in the simulations.

For the first 50 years of the low simulation, *A. altissima* (height: 9.29±0.09 m) and *A. rubrum* (height: 7.53±0.12 m) were the largest trees (Fig. 6). After 100 years of the simulation, the species occupying the canopy included *A. altissima* (46%), *A. rubrum* (22%), *C. glabra* (15%), and *P. serotina* (9%). At this time the tallest tree in the canopy of the forest was 24.5 m, and trees classified as canopy trees included all trees three meters below this height. At the end of the simulation *A. altissima* (51%) was dominant among the other canopy species: *A. rubrum* (23%), *C. glabra* (15%), and *P. serotina* (9%) (Fig. 7a). The canopy height at the end of the simulation ranged from 25.6-28.6 m.

When moderate levels of O_3 were modeled, *A. rubrum* was the largest species throughout the entire simulation (Fig. 6), though *A. altissima* (30%) remained a canopy species. Rather than reaching the top of the canopy, *P. serotina* remained an understory tree species. The late-successional *Carya* completely dropped out of the canopy potentially due to its O_3 sensitivity. After 100 years, the tallest tree in the canopy was 18.1 m tall, and the canopy was composed of *A. rubrum* (48%) and *A. altissima* (47%). By the end of the simulation the canopy reached 23.1 m, and still consisted largely of *A. rubrum* (64%) and *A. altissima* (30%) (Fig. 7b). The group of native species (height: 6.20±0.05 m, DBH: 9.66±0.16 cm) was consistently larger than the group of invasive species (height: 4.00±0.10 m, DBH: 7.80±0.17 cm).

In the high simulation, the rank of species size distribution was similar to the species characteristics measured in the low simulation, but trees were smaller on average in the high simulation (height: 5.21±0.08 m) (Fig. 8c). Though many similarities existed between the high and low forests, the high O_3 forest had a shorter canopy throughout the

simulation, though it was taller than the medium O_3 forest. *Carya glabra* appeared later in the high O_3 forest canopy (~170 years) than the low O_3 forest (~120 years) suggesting a potential delay caused in succession due to O_3 stress. Throughout the entire simulation, native and invasive species did not differ in size distribution.

Overall forest structure

A Pearson product-moment correlation test found a strong positive relationship existed throughout all trials between simulated tree height and diameter (Table 4), which resulted from the allometric functions embedded within the model. The abundance of trees was weakly negatively correlated with height and DBH likely due to the high abundance of very short saplings. As the simulation progressed these correlations became more significant. On average, height growth rates were more rapid early (0-100 years) in the simulation while the diameter growth rates remained steady throughout the simulations.

At low O_3 levels (<80 ppb), trees grew tallest and largest in diameter (Fig. 8a). The lower density of trees (4922±62 ha^{-1}) allowed for high resource allocation, which led to larger sized trees. After canopy closure (~80 years), the large trees successfully shaded out seedlings and acquired resources better than younger trees, leading to high mortality of seedlings and saplings. The low O_3 forest produced the highest woody biomass (475.53±5.99 MG ha^{-1}) of all simulations.

Trees in the medium O_3 forest were shorter and smaller, and less abundant than any other simulation (Fig. 8b). Tree abundance decreased with forest age, and at year

200 the number of trees alive in one hectare was 3976±155, significantly lower than the low simulation. The large total number of simulated trees in the medium O_3 forest (n=82,584±3219) relative to the other treatments, and the low abundance of trees in any given year suggest a high mortality rate in the medium simulation. This forest was characterized by the lowest woody biomass (223.10±8.68 MG ha^{-1}) among all treatments. The size distribution at the end of the simulation was similar to the low treatment for trees larger than 25 cm in diameter, but had ten percent less intermediate sized trees (i.e., 5-25 cm) and eleven percent more small trees (i.e., <5 cm). This pattern was likely regulated by the high mortality of mature trees.

The high simulation had significantly smaller trees than the low simulation, but these trees were larger than those in the medium treatment. During the first 50 years of the simulation, medium treatment trees were larger than those in the high O_3 simulation, but this changed after 100 years of forest growth. The early stages of forest growth in the high O_3 simulation were marked by a high mortality of seedlings and saplings compared to the medium treatment (Fig. 8). The total number of trees simulated was the lowest for all simulations (n=47,864±440) while 5055±46 trees per hectare remained after 200 years of simulation. These values suggest the high O_3 treatment had the lowest mortality rate across O_3 treatments.

Discussion

The use of TREGRO integrated with ZELIG demonstrated that elevated O_3 is capable of altering species composition, size distribution, and overall forest structure. Patterns of forest structure and species composition were driven by sensitivity to O_3 and shade. Species composition differed for each treatment by the first 50 years of the simulations. By the end of the low O_3 forest simulation, increases of two fast growing invasive species (*Ailanthus altissima*, *Quercus acutissima*) accompanied a decline in one native species (*Prunus serotina*). A different pattern of species response was displayed in the medium treatment where one O_3 tolerant native species (*Acer rubrum*) increased in size and abundance. This O_3 tolerant native species replaced several invasive species (*Ailanthus altissima*, *Quercus acutissima*) early in the simulation. A number of other O_3 tolerant native species (*Prunus serotina*, *Celtis occidentalis*) also increased in abundance. While low and high treatments had similar species composition, the canopies of the forests differed in percent of co-dominating canopy species and in overall size (i.e., low treatment trees were larger than high treatment trees). Medium O_3 stress resulted in the highest abundance of native species, but smallest tree size, highest mortality, and the least dense forest. The high O_3 forest had higher average tree size and higher average canopy height than the medium treatment.

Forest species dynamics

The diversity of tree species increased as O_3 concentrations rose from low to high. Diversity was high for all treatments during the first 50 years while species experienced

low levels of competition due to high resource availability. However, diversity dropped precipitously in the low O_3 treatment simulations as competition increased with the closing of the canopy.

As the forests continued to grow, species diversity in the low treatment decreased, mirroring patterns typically seen in Mid-Atlantic forests as resources become limited (Huston 1994) and canopy closure occurs (Elliott and Swank 1994). During later successional stages, competition for light is more intense than competition in early succession when plants are competing for soil resources (Tilman 1985). With increasing competition for resources, diversity was expected to decrease due to competitive exclusion (Huston 1979, Gould 1989). The dominance of a couple of species limited diversity in the low O_3 treatment and greatly reduced diversity as competition increased. Unlike the low treatment, diversity remained high throughout the entire simulation for the medium and high O_3 forests. As described by Grime's "humped-back" model of species richness (1973), competitive species are unable to outcompete others when stress levels increase, often leading to increased species diversity. It is unlikely that further increases in O_3 concentrations would cause major reductions in species diversity. The maintenance of high diversity in the high O_3 treatment resulted from reductions in the abundance of three dominant O_3 sensitive species (*Acer rubrum*, *Celtis occidentalis*, *Ailanthus altissima*) and the increase in abundance of three O_3 and shade tolerant species (*Carya glabra*, *Paulownia tomentosa*, *Quercus acutissima*). Whether this pattern is generally the case would depend on whether the dominant species in a forest tend to be O_3 sensitive.

The range of O_3 sensitivity among species resulted in forests of varying composition. High O_3 sensitivity of a species predicted reductions in abundance. For example, when comparing low to medium O_3 forests, species with high O_3 sensitivity decreased in abundance (*C. glabra*, *Q. acutissima*, *Quercus alba*). Increases in abundance were a result of both O_3 and shade tolerance since the majority of O_3 sensitivity reductions occurred after canopy closure. The species that took advantage of the reduction in tree density were O_3 and shade tolerant species (*C. occidentalis*, *P. serotina*, *A. rubrum*). Shade tolerant trees often recolonize gaps in closed canopy forests due to their ability to maintain higher growth rates before the opening of gaps (White 1979, Connell 1989, Whitmore 1989, Gray and Spies 1997).

These simulations were some of the first model results to indicate the potential of O_3 to affect species composition. Even though large changes in basal area were found for both *L. tulipifera* and *Pinus taeda* in a ZELIG simulation evaluating the effects of increased O_3, these changes did not create shifts in species composition (Laurence et al. 2001). By including O_3 tolerance information for only one to two species of interest, shifts in species interactions are limited. The inclusion of previously quantified species-specific O_3 tolerance information (Elton Chapter 1) for the 13 study species increased the potential for composition shifts related to O_3 stress.

When evaluating the success of species in these trials, it is important to remember that the simulations began with bare ground, analogous to the abandonment of an agricultural field. These conditions favored pioneer species that were typically shade intolerant and had high seed production. Under mature forest conditions these pioneer

species require the creation of canopy gaps or edges to establish (Knapp and Canham 2000). In this study, small gaps were created from O_3 stress, which supported the persistence of pioneer species even after late-successional species entered the canopy.

This study included native pioneer species (*A. rubrum*, *C. occidentalis*, *R. pseudoacacia*, *L. tulipifera*, *P. serotina*) that share similar life history traits with invasive species. Typically, invasive species outperform native species in high stress environments (Cleverly et al. 1997, Alpert et al. 2000). However, native pioneer species are able to colonize and grow under highly stressed conditions, which promoted the success of *A. rubrum* as a major canopy species in the majority of trials in this study.

Forest structure

Despite shifts in species composition across O_3 treatments, the same group of species remained as canopy species in nearly every simulation. The model favored pioneer species, allowing them to establish initially as canopy species and readily fill gaps created by O_3 stress. Small shifts in canopy structure did occur in response to O_3 sensitivity. For example, in the medium treatment, the O_3 tolerant *A. rubrum* seemed to occupy the space left by the O_3 sensitive *C. glabra* and *P. serotina*. The success of *A. rubrum* in achieving canopy height before other species in the simulation likely disadvantaged the previously dominant *A. altissima* due to its shade intolerance. During the high simulation, *C. glabra* reclaimed a portion of the canopy as its O_3 tolerance increased and *A. rubrum* suffered increases in O_3 sensitivity.

The native oak species (*Q. alba* and *Q. rubra*) were initially expected to reach canopy height in the model due to their dominance in Mid-Atlantic forests. It is unrealistic, however, to assume the modeled conditions would reflect the succession history of this region. One noteworthy cause of the absence of native *Quercus* spp. from the canopy could be disturbance intensity. For example, *Q. alba* and *Q. rubra* are known to dominate canopies with frequent fire recurrence as shown in Mettler's Woods, New Jersey where a fire interval of 14 years created an environment where *Quercus* dominance is higher than most other areas in the Mid-Atlantic region (Buell et al. 1954). A combination of O_3 sensitivity and the lack of major disturbances in the simulations resulted in *Quercus* spp. remaining within the understory throughout the duration of the simulations.

Average tree size was largest for the low O_3 simulation as expected, due to reduced stress on tree growth (Wittig et al. 2007). Ozone was expected to cause a decrease in size as O_3 concentrations rose (Cooley and Manning 1987, Darbah et al. 2007, Grams and Matyssek 2010), but tree size was the smallest in the medium treatment. The larger mean tree size in the high O_3 forest relative to the medium O_3 forest was likely due to two factors. First, the medium O_3 levels caused marked reductions in one of the dominant species (*Q. acutissima*), which allowed other previously suppressed, more fecund species to reach maturity. The increase in the number of seeds in the seedbank led to a higher number of germinating seeds in the medium treatment. This increase in seedling density caused increased competition for resources within this size class compared to the less dense high O_3 forest. When a gap opened in the medium O_3 forest,

the highly competitive cohort of seedlings responded slowly due to limited resources. The low tree density in the high O_3 forest reduced competition allowing surviving trees to grow larger. Secondly, the high mortality of intermediate sized trees (i.e., 10-25 cm in DBH) occurring within the first 100 years of the medium O_3 forest reduced mean size in this simulation. High levels of O_3 stress suppressed the numbers of seedlings and saplings after canopy closure. This allowed these surviving seedling and saplings to acquire resources from a larger area than trees in the medium simulation. The promotion of growth resulting from high resource availability in the high O_3 forest allowed small trees to reach intermediate size more successfully than trees in the medium treatment. A long history of silviculture practices have used sapling thinning to reduce tree density in early aged stands to increase annual growth (Bohmer 1957, Andreassen 1994, Andreassen 1995, Lundqvist et al. 2007).

Conclusions

The differential sensitivity to O_3 and shade among tree species produced shifts in species composition and forest structure throughout the three O_3 forest simulations. However, increased exposure to O_3 stress did not result in increased dominance of invasive species. Increased diversity was found at the medium and high O_3 treatment due to the O_3 sensitivity of a number of dominant species, which allowed more rare species to increase in abundance.

The simulation conditions favored pioneer species, which led to a group of fast growing native and invasive species dominating the canopy throughout all simulations.

While canopy dominance was maintained by a small group of species, the proportion of these species found in the canopy depended upon the sensitivity of each species to O_3. The largest trees were found in the low O_3 forest, likely a result of reduced oxidative stress. The high O_3 forest was smaller than the low O_3 forest, but reduced competition in the high O_3 forest among seedlings resulted in larger trees than the medium O_3 forest.

Information from this study may prove useful to forest managers in regulating damage caused by O_3 and the invasion of non-natives in areas with elevated ground-level O_3. Early growth stage thinning found in the high treatment could reduce stress allowing for higher rates of growth and biomass accumulation in areas of elevated ground-level O_3. In order to best protect against invasive species, this study suggests that land managers focus on maintaining healthy populations of shade and O_3 tolerant native species.

To improve model simulations, future studies should focus on obtaining O_3 tolerance information for a number of other species found to be of importance in areas that currently experience periodic elevated O_3 stress. This study included 13 of 54 tree species found in previously surveyed forests (Elton Chapter 1). The inclusion of more species-specific O_3 sensitivity information within linked models will allow for more robust predictions of forest dynamics in the Mid-Atlantic forest community.

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Literature Cited

Abrams, M D. 1992. Fire and the development of oak forests in eastern North America, oak distribution reflects a variety of ecological paths and disturbance conditions. *Bioscience* 42: 346-353.

Abrams, M D and G J Nowacki. 1992. Historical variation in fire, oak recruitment, and post-logging accelerated succession in central Pennsylvania. *Bulletin of the Torrey Botanical Club* 119: 19-28.

Alpert, P, E Bone, and C Holzapfel. 2000. Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in Plant Ecology, Evolution and Systematics* 3: 52-66.

Andreassen, K. 1995. Long-Term experiments in selectively cut Norway spruce (*Picea abies*) forest. *Water, Air, & Soil Pollution* 82: 97-105.

Andreassen, K. 1994. Development and yield in selection forest. *Meddelelser Fra Skogforsk (Norway); Communications of Skogforsk*.

Angilletta Jr, M J. 2009. Looking for answers to questions about heat stress: Researchers are getting warmer. *Functional Ecology* 23: 231-232.

Ashton, I W, L A Hyatt, K M Howe, J Gurevitch, and M T Lerdau. 2005. Invasive species accelerate decomposition and litter nitrogen loss in a mixed deciduous forest. *Ecological Applications* 15: 1263-1272.

Baek, S G and S Y Woo. 2010. Physiological and biochemical responses of two tree species in urban areas to different air pollution levels. *Photosynthetica* 48: 23-29.

Baker, C K, J J Colls, A E Fullwood, and G G R Seaton. 1986. Depression of growth and yield in winter barley exposed to sulphur dioxide in the field. *New Phytologist* 104: 233-241.

Bartholomay, G A, R T Eckert, and K T Smith. 1997. Reductions in tree-ring widths of white pine following ozone exposure at Acadia National Park, Maine, USA. *Canadian Journal of Forest Research* 27: 361-368.

Bassin, S, R A Werner, K Sörgel, M Volk, N Buchmann, and J Fuhrer. 2009. Effects of combined ozone and nitrogen deposition on the in situ properties of eleven key plant species of a subalpine pasture. *Oecologia* 158: 747-56.

Bassow, S L and F A Bazzaz. 1997. Intra- and inter-specific variation in canopy photosynthesis in a mixed deciduous forest. *Oecologia* 109: 507-515.

Bella, I E. 1971. A new competition model for individual trees. *Forest Science* 17: 364-372.

Beresford-Jones, D, H Lewis, and S Boreham. 2009. Linking cultural and environmental change in Peruvian prehistory: Geomorphological survey of the Samaca Basin, Lower Ica Valley, Peru. *Catena* 78: 234-249.

Bohmer, A J G. 1957. Growth of forests II: Practices. *Tidsskrift for Skogbruk:* 203.

Botkin, D B, J F Janak, and J R Wallis. 1972. Some ecological consequences of a computer model of forest growth. *The Journal of Ecology* 60: 849-872.

Braun, E L. 1950. Deciduous forests of the eastern United States. *Hafner Publ. Co. New York.*

Buell, M F. 1957. The mature oak forest of Mettler's woods. *William Hutcheson L. Memorial Forest Bulletin* 1: 16-19.

Buell, M F, H F Buell, and J A Small. 1954. Fire in the history of Mettler's woods. *Bulletin of the Torrey Botanical Club* 81: 253-255.

Busing, R T. 1995. Disturbance and the population dynamics of *Liriodendron tulipifera*: Simulations with a spatial model of forest succession. *The Journal of Ecology* 83: 45-53.

Christensen, N L. 1977. Changes in structure, pattern and diversity associated with climax forest maturation in piedmont, North Carolina. *American Midland Naturalist* 97: 176-188.

Cleverly, J R, S D Smith, A Sala, and D A Devitt. 1997. Invasive capacity of *Tamarix ramosissima* in a Mojave Desert floodplain: The role of drought. *Oecologia* 111: 12-18.

Connell, J H. 1989. Some processes affecting the species composition in forest gaps. *Ecology* 70: 560-562.

Cooley, D R and W J Manning. 1987. The impact of ozone on assimilate partitioning in plants: A review. *Environmental Pollution* 47: 95-113.

Cumming, S G and P J Burton. 1996. Phenology-Mediated effects of climatic change on some simulated British Columbia forests. *Climatic Change* 34: 213-222.

D'Antonio, C M. 2000. *Fire, Plant Invasions, and Global Changes. Mooney HA & RJ Hobbs (Eds) Invasive Species in a Changing World: 65-93.* Island Press, Covelo, California.

D'Antonio, C M and P M Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annual Review of Ecology and Systematics* 23:63-87.

D'Antonio, C M and B E Mahall. 1991. Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. *American Journal of Botany* 78: 885-894.

Darbah, J N T, M E Kubiske, N Nelson, E Oksanen, E Vaapavuori, and D F Karnosky. 2007. Impacts of elevated atmospheric CO_2 and O_3 on paper birch (*Betula papyrifera*): Reproductive fitness. *Scientific World Journal* 7: 240-6.

Day, G M. 1953. The indian as an ecological factor in the northeastern forest. *Ecology* 34: 329-346.

Dentener, F, S Kinne, T Bond, O Boucher, J Cofala, S Generoso, P Ginoux, S Gong, J J Hoelzemann, and A Ito. 2006. Emissions of primary aerosol and precursor gases in the years 2000 and 1750 prescribed data-sets for aerocom. *Atmospheric Chemistry and Physics* 6: 4321-4344.

Dittmar, C, W Zech, and W Elling. 2003. Growth variations of common beech (*Fagus sylvatica* L.) Under different climatic and environmental conditions in Europe--a dendroecological study. *Forest Ecology and Management* 173: 63-78.

Dunson, W A and J Travis. 1991. The role of abiotic factors in community organization. *American Naturalist* 138: 1067-1091.

Ehrenfeld, J G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6: 503-523.

Ehrenfeld, J G. 1997. Invasion of deciduous forest preserves in the New York metropolitan region by Japanese barberry (*Berberis thunbergii* DC.). *Journal of the Torrey Botanical Society* 124: 210-215.

Ek, A R and R A Monserud. 1974. *FOREST: A Computer Model for Simulating the Growth and Reproduction of Mixed Species Forest Stands.* School of Natural Resources, College of Agricultural and Life Sciences, University of Wisconsin.

Elliott, K J and W T Swank. 1994. Changes in tree species diversity after successive clearcuts in the southern Appalachians. *Plant Ecology* 115: 11-18.

FAO. 2010. Global forest resources assessment 2010.FAO Forestry Paper

FAO. 2006. Global forest resources assessment 2005.Progress Towards Sustainable Forest Management

Forman, R T T and B A Elfstrom. 1975. Forest structure comparison of Hutcheson Memorial Forest and eight old woods on the New Jersey piedmont. *William L. Hutcheson Memorial Forest Bull* 3: 44-51.

Foster, D R, J D Aber, J M Melillo, R D Bowden, and F A Bazzaz. 1997. Forest response to disturbance and anthropogenic stress. *Bioscience* 47: 437-445.

Fowler, D, M Amann, R Anderson, M Ashmore, P Cox, M Depledge, D Derwent, P Grennfelt, N Hewitt, and O Hov. 2008. Ground-Level ozone in the 21st century: Future trends, impacts and policy implications. *The Royal Society Policy Document* 132.

Fowler, D, J N Cape, M Coyle, C Flechard, J Kuylenstierna, K Hicks, D Derwent, C Johnson, and D Stevenson. 1999a. The global exposure of forests to air pollutants. *Water, Air, & Soil Pollution* 116: 5-32.

Fowler, D, J N Cape, M Coyle, R I Smith, A G Hjellbrekke, D Simpson, R G Derwent, and C E Johnson. 1999b. Modelling photochemical oxidant formation, transport, deposition and exposure of terrestrial ecosystems. *Environmental Pollution* 100: 43-55.

Fox, M D and B J Fox. 1986. The effect of fire frequency on the structure and floristic composition of a woodland understory. *Australian Journal of Ecology. Oxford* 11: 77-85.

Friend, A D, H H Shugart, and S W Running. 1993. A physiology-based gap model of forest dynamics. *Ecology* 74: 792-797.

Gerlach, J D. 2000. *A Model Experimental System for Predicting the Invasion Success and Ecosystem Impacts of Non-Indigenous Summer-Flowering Annual Plants in California's Central Valley Grasslands and Oak Woodlands.* University of California, Davis.

Girgzdiene, R, B Serafinaviciute, V Stakenas, and S Bycenkiene. 2009. Ambient ozone concentration and its impact on forest vegetation in Lithuania. *Ambio* 38: 432-436.

Gould, S J. 1989. Punctuated equilibrium in fact and theory. *Journal of Social and Biological Structures* 12: 117-136.

Gómez-Aparicio, L and C D Canham. 2008. Neighborhood analyses of the allelopathic effects of the invasive tree *Ailanthus altissima* in temperate forests. *Journal of Ecology* 96: 447-458.

Grams, T E E. 2010. Stable isotope signatures reflect competitiveness between trees under changed CO_2/O_3 regimes. *Environmental Pollution* 158: 1036-1042.

Grams, T E E, A R Kozovits, K H Haberle, R Matyssek, and T E Dawsom. 2007. Combining δ13c and δ18o analyses to unravel competition, CO_2 and O_3 effects on the physiological performance of different-aged trees. *Plant Cell and Environment* 30: 1023-1034.

Grams, T E E and R Matyssek. 1999. Elevated CO_2 counteracts the limitation by chronic ozone exposure on photosynthesis in *Fagus sylvatica* L.: Comparison between chlorophyll fluorescence and leaf gas exchange. *Phyton* 39: 31-40.

Gray, A N and T A Spies. 1997. Microsite controls on tree seedling establishment in conifer forest canopy gaps. *Ecology* 78: 2458-2473.

Grime, J P. 1973. Competitive exclusion in herbaceous vegetation. *Nature* 242:344-347.

Harrison, E A and H H Shugart. 1990. Evaluating performance of an Appalachian oak forest dynamics model. *Plant Ecology* 86: 1-13.

He, X Y, Y N Ruan, W Chen, and T Lu. 2006. Responses of anti-oxidative system in leaves of *Ginkgo biloba* to elevated ozone concentration in urban area. *Botanical Studies* 47: 409-416.

Heisey, R M. 1990. Evidence for allelopathy by tree-of-heaven (*Ailanthus altissima*). *J Chemical Ecology* 16: 2039-2055.

Hobbs, R J. 1989. The nature and effects of disturbance relative to invasions. *Biological Invasions: A Global Perspective* 389-405.

Holmes, P M and R M Cowling. 1997. The effects of invasion by *Acacia saligna* on the guild structure and regeneration capabilities of South African fynbos shrublands. *Journal of Applied Ecology* 34: 317-332.

Huston, M. 1979. A general hypothesis of species diversity. *American Naturalist* 113: 81-101.

Karnosky, D F, K S Pregitzer, D R Zak, M E Kubiske, G R Hendrey, D Weinstein, M Nosal, and K E Percy. 2005. Scaling ozone responses of forest trees to the ecosystem level in a changing climate. *Plant Cell and Environment* 28: 965.

Karnosky, D F, D R Zak, K S Pregitzer, C S Awmack, J G Bockheim, R E Dickson, G R Hendrey, G E Host, J S King, and B J Kopper. 2003. Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: A synthesis of molecular to ecosystem results from the aspen FACE project. *Functional Ecology* 17: 289-304.

Kay, M. 1994. Biological control for invasive tree species. *New Zealand Forestry* 39: 35-37.

Knapp, L B and C D Canham. 2000. Invasion of an old-growth forest in New York by *Ailanthus altissima*: Sapling growth and recruitment in canopy gaps. *Journal of the Torrey Botanical Society* 127: 307-315.

Kozlowski, T T, P J Kramer, and S G Pallardy. 1991. The physiological ecology of woody plants. *Tree Physiology* 8: 213.

Krupa, S V, M Nosal, and A H Legge. 1998. A numerical analysis of the combined open-top chamber data from the USA and Europe on ambient ozone and negative crop responses. *Environmental Pollution* 101: 157-160.

Lake, J C and M R Leishman. 2004. Invasion success of exotic plants in natural ecosystems: The role of disturbance, plant attributes and freedom from herbivores. *Biological Conservation* 117: 215-226.

Lambers, H, F S Chapin, and T L Pons. 1998. *Plant Physiological Ecology.* Springer.

Larocque, G R. 2010. Development of the gap model ZELIG-CFS to predict the dynamics of North American mixed forest types with complex structures. *Ecological Modelling*. *In press.*

Larocque, G R, J S Bhatti, R Boutin, and O Chertov. 2008. Uncertainty analysis in carbon cycle models of forest ecosystems: Research needs and development of a theoretical framework to estimate error propagation. *Ecological Modelling* 219: 400-412.

Larocque, G R . 2007. Validating the gap model ZELIG for an uneven-aged mixedwood forest type in southeastern Canada. *Complex Stand Structures and Associated Dynamics: Measurement Indices and Modelling* 85.

Larocque, G R, L Archambault, and C Delisle. 2006. Modelling forest succession in two southeastern Canadian mixedwood ecosystem types using the ZELIG model. *Ecological Modelling* 199: 350-362.

Laurence, J A and C P Andersen. 2003. Ozone and natural systems: Understanding exposure, response, and risk. *Environ International* 29: 155-160.

Laurence, J A, W A Retzlaff, J S Kern, E H Lee, W E Hogsett, and D A Weinstein. 2001. Predicting the regional impact of ozone and precipitation on the growth of loblolly pine and yellow-poplar using linked TREGRO and ZELIG models. *Forest Ecology and Management* 146: 247-263.

Laurence, J A, R J Kohut, and R G Amundson. 1993. Use of TREGRO to simulate the effects of ozone on the growth of red spruce seedlings. *Forest Science* 39: 453-464.

Lawrence, J G, A Colwell, and O J Sexton. 1991. The ecological impact of allelopathy in *Ailanthus altissima* (simaroubaceae). *American Journal of Botany* 78: 948-958.

Lee, Y. 1967. *Stand Models for Lodgepole Pine and Limits to Their Application.* University of British Columbia.

Lefohn, A S and J K Foley. 1992. NCLAN results and their application to the standard-setting process: Protecting vegetation from surface ozone exposures. National crop loss assessment network. *Journal of the Air & Waste Management Association* 42: 1046.

Lichstein, J W, H R Grau, and R Aragón. 2004. Recruitment limitation in secondary forests dominated by an exotic tree. *Journal of Vegetation Science* 15: 721-728.

Little, S. 1974. Effects of fire on temperate forests: Northeastern United States. *Fire and Ecosystems* 225-250.

Long, R P and D D Davis. 1991. Black-Cherry growth-response to ambient ozone and EDU. *Environ Pollution* 70: 241-254.

Loope, L L, W Sanchez Peter, and G Peter. 1988. Biological invasions of arid land nature reserves. *Biological Conservation* 44: 95-118.

Luedemann, G, R Matyssek, J B Winkler, and T E E Grams. 2009. Contrasting ozone x pathogen interaction as mediated through competition between juvenile European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*). *Plant and Soil* 323:47-60.

Lundqvist, L, D Chrimes, B Elfving, T Mörling, and E Valinger. 2007. Stand development after different thinnings in two uneven-aged *Picea abies* forests in Sweden. *Forest Ecology and Management* 238: 141-146.

Mack, M C and C M D'Antonio. 1998. Impacts of biological invasions on disturbance regimes. *Trends in Ecology and Evolution* 13: 195-198.

Marcano-Vega, H, T Mitchell Aide, and D Báez. 2002. Forest regeneration in abandoned coffee plantations and pastures in the cordillera central of Puerto Rico. *Plant Ecology* 161: 75-87.

Marenco, A, H Gouget, P Nédélec, J P Pagés, and F Karcher. 1994. Evidence of a long-term increase in tropospheric ozone from Pic du Midi data series: Consequences: Positive radiative forcing. *Journal of Geophysical Research* 99 (D8): 16617.

Martin, P H. 1999. Norway maple (*Acer platanoides*) invasion of a natural forest stand: Understory consequence and regeneration pattern. *Biological Invasions* 1: 215-222.

Matyssek, R, D F Karnosky, G Wieser, K Percy, E Oksanen, T E E Grams, M Kubiske, D Hanke, and H Pretzsch. 2010. Advances in understanding ozone impact on forest trees: Messages from novel phytotron and free-air fumigation studies. *Environmental Pollution* 158: 1990-2006.

Mazia, C N, E J Chaneton, C M Ghersa, and R J C Leon. 2001. Limits to tree species invasion in Pampean grassland and forest plant communities. *Oecologia* 128: 594-602.

McDowell, N, W T Pockman, C D Allen, D D Breshears, N Cobb, T Kolb, J Plaut, J Sperry, A West, and D G Williams. 2008. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytologist* 178: 719-739.

Melgoza, G, R S Nowak, and R J Tausch. 1990. Soil water exploitation after fire: Competition between *Bromus tectorum* (cheatgrass) and two native species. *Oecologia* 83: 7-13.

Mitchell, K J. 1969. *Simulation of the Growth of Even-Aged Stands of White Spruce.* Yale University.

Munro, D D. 1974. Forest growth models--a prognosis. Growth models for tree and stand simulation. In *Proceedings of the IUFRO Congress S.*

Musselman, R C, A S Lefohn, W J Massman, and R L Heath. 2006. A critical review and analysis of the use of exposure-and flux-based ozone indices for predicting vegetation effects. *Atmospheric Environment* 40: 1869-1888.

Naeem, S, J M H Knops, D Tilman, K M Howe, T Kennedy, and S Gale. 2000. Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. *OIKOS* 91: 97-108.

Newham. 1964. *The development of a stand model for Douglas-fir*. University of British Colombia.

Novak, S J. 2007. The role of evolution in the invasion process. *Proceedings of the National Academy of Sciences* 104: 3671.

Nowacki, G J and M D Abrams. 1991. Community and edaphic analysis of mixed oak forests in the ridge and valley province of central Pennsylvania. *8th Central Hardwood Forest Conference*.

Ollinger, S V, J D Aber, and A Federer. 1998. Estimating regional forest productivity and water yield using an ecosystem model linked to a GIS. *Landscape Ecology* 13: 323-334.

Orendovici-Best, T, J M Skelly, D D Davis, J A Ferdinand, J E Savage, and R E Stevenson. 2008. Ozone uptake (flux) as it relates to ozone-induced foliar symptoms of *Prunus serotina* and *Populus maximowizii× trichocarpa*. *Environmental Pollution* 151: 79-92.

Pabst, R J, M N Goslin, S L Garman, and T A Spies. 2008. Calibrating and testing a gap model for simulating forest management in the Oregon coast range. *Forest Ecological Management* 256: 958-972.

Pacala, S W, and G C Hurtt. 1993. Terrestrial vegetation and climate change: Integrating models and experiments. *Biotic Interactions and Global Change* 57-74.

Patton, D R. 1992. Wildlife habitat relationships in forested ecosystems, Timber Press Inc., Portland, Oregon, 392.

Prozherina, N, E Nakvasina, and E Oksanen. 2009. Impact of experimentally elevated ozone on seed germination and growth of Russian pine (*Pinus sylvestris*) and spruce (*Picea* spp.) Provenances. *Ambio* 38: 443-447.

Retzlaff, W A, M A Arthur, N E Grulke, D A Weinstein, and B Gollands. 2000. Use of a single-tree simulation model to predict effects of ozone and drought on growth of a white fir tree. *Tree Physiology* 20: 195-202.

Robertson, D J, M C Robertson, and T Tague. 1994. Colonization dynamics of four exotic plants in a northern piedmont natural area. *Bulletin of the Torrey Botanical Club* 121: 107-118.

Rolett, B and J Diamond. 2004. Environmental predictors of pre-European deforestation on pacific islands. *Nature* 431: 443-6.

Rubin, G, C E McCulloch, and J A Laurence. 1996. A model for estimating daily ozone dose for plants from atmospheric ozone concentration and vapor pressure deficit. *Journal of Agricultural, Biological, and Environmental Statistics* 1: 1-16.

Russell, E W B. 1980. Vegetational change in northern New Jersey from precolonization to the present: A palynological interpretation. *Bulletin of the Torrey Botanical Club* 107: 432-446.

Sakai, A K, F W Allendorf, J S Holt, D M Lodge, J Molofsky, K A With, S Baughman, R J Cabin, J E Cohen, and N C Ellstrand. 2001. The population biology of invasive species. *Annual Review of Ecology and Systematics* 32: 305-332.

Samuelson, L J and G S Edwards. 1993. A comparison of sensitivity to ozone in seedlings and trees of *Quercus rubra* L. *New Phytologist* 125: 373-379.

Seabloom, E W, W S Harpole, O J Reichman, and D Tilman. 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proceedings of the National Academies Sciences USA* 100: 13384-13389.

Seischab, F K and D Orwig. 1991. Catastrophic disturbances in the presettlement forests of western New York. *Bulletin of the Torrey Botanical Club* 118: 117-122.

Shugart, H H .1984. *A Theory of Forest Dynamics: The Ecological Implications of Forest Succession Models.* Springer-Verlag, New York.

Shugart, H H. 1980. Environmental gradients in a simulation model of a beech-yellow-poplar stand. *Mathematical Biosciences* 50: 163-170.

Shugart, H H and D C West. 1977. Development of an Appalachian deciduous forest succession model and its application to assessment of the impact of the chestnut blight. *Journal of Environmental Management* 5: 161-180.

Smith, T and M Huston. 1989. A theory of the spatial and temporal dynamics of plant communities. *Plant Ecology* 83: 49-69.

Smith, T M and D L Urban. 1988. Scale and resolution of forest structural pattern. *Plant Ecology* 74: 143-150.

Smith, W B, P D Miles, J S Vissage, and S A Pugh. 2003. Forest resources of the United States, 2002. USDA Forest Service, North Central Research Station. *General Technical Report, NC-241*137.

Solomon, A M. 1986. Transient response of forests to CO_2-induced climate change: Simulation modeling experiments in eastern North America. *Oecologia* 68: 567-579.

Solomon, A M and T Webb III. 1985. Computer-Aided reconstruction of late-quaternary landscape dynamics. *Annual Review of Ecology and Systematics* 16:63-84.

Solomon, A M and H H Shugart. 1983. Integrating forest-stand simulations with paleoecological records to examine long-term forest dynamics Oak Ridge National Lab., TN (USA).

Solomon, A M, D C West, and J A Solomon. 1981. Simulating the role of climate change and species immigration in forest succession. *Forest Succession: Concepts and Application* 154-177.

Solomon, A M, H R Delcourt, D C West, and T J Blasing. 1980. Testing a simulation model for reconstruction of prehistoric forest-stand dynamics. *Quaternary Research* (Ny); (United States) 14.

Somers, G L, A H Chappelka, P Rosseau, and J R Renfro. 1998. Empirical evidence of growth decline related to visible ozone injury. *Forest Ecology and Management* 104: 129-137.

Stephenson, S L. 1986. Changes in a former chestnut-dominated forest after a half century of succession. *American Midland Naturalist* 116: 173-179.

Tilman, D. 1985. The resource-ratio hypothesis of plant succession. *American Naturalist* 125: 827-852.

Tingey, D T, J A Weber, J Greene, W E Hogsett, E H Lee, J A Laurence, and S Brown. 2001. Elevated CO_2 and temperature alter the response of *Pinus ponderosa* to ozone: A simulation analysis. *Ecological Applications* 11: 1412-1424.

Tunison, J T, R Loh, and C D'Antonio. 2001. Fire, grass invasions and revegetation of burned areas in Hawaii volcanoes national park. In *Proceedings of the Invasive Species Workshop: The Role of Fire in the Control and Spread of Invasive Species.* Tall Timbers Research Station Publication.

Tylianakis, J M, R K Didham, J Bascompte, and D A Wardle. 2008. Global change and species interactions in terrestrial ecosystems. *Ecology Letters* 11: 1351-1363.

Urban, D L, G B Bonan, T M Smith, and H H Shugart. 1991. Spatial applications of gap models. *Forest Ecology and Management* 42: 95-110.

Urban, D L. 1990. A versatile model to simulate forest pattern: A user's guide to ZELIG version 1.0. *Environmental Sciences Department, the University of Virginia, Charlottesville, Virginia* 107.

Urban, D L, H H Shugart, D Smith, and D Tirpak. 1989. The Potential Effects of Global Climate Change on the United States: Appendix D –Forests [Smith, J B and D A Tirpak (eds.)]. US Environmental Protection Agency, Washington, DC, USA, 176.

Vinebrooke, R D, K L Cottingham, J Norberg, M Scheffer, S I Dodson, S C Maberly, and U Sommer. 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: The role of species co-tolerance. *Oikos* 104: 451-457.

Vingarzan, R. 2004. A review of surface ozone background levels and trends. *Atmospheric Environment* 38: 3431-3442.

Vitousek, P M. 1990. Biological invasions and ecosystem processes: Towards an integration of population biology and ecosystem studies. *Oikos* 57: 7-13.

Vitousek, P M, L L Loope, and C P Stone. 1987. Introduced species in Hawaii. Biological effects and opportunities for ecological research. *Trends in Ecology and Evolution* 2: 224-227.

Wang, M, D-X Guan, S-J Han, and J-L Wu. 2010. Comparison of eddy covariance and chamber-based methods for measuring CO_2 flux in a temperate mixed forest. *Tree Physiology* 30: 149-63.

Weinstein, D A, J A Laurence, W A Retzlaff, J S Kern, E H Lee, W E Hogsett, and J Weber. 2005. Predicting the effects of tropospheric ozone on regional productivity of ponderosa pine and white fir. *Forest Ecology and Management* 205: 73-89.

Weinstein, D A, B Gollands, and W A Retzlaff. 2001. The effects of ozone on a lower slope forest of the Great Smoky Mountain National Park: Simulations linking an individual tree model to a stand model. *Forest Science* 47: 29-42.

Weinstein, D A and R D Yanai. 1994. Integrating the effects of simultaneous multiple stresses on plants using the simulation model TREGRO. *Journal of Environmental Quality* 23: 418-428.

Weinstein, D A, R M Beloin, and R D Yanai. 1991. Modeling changes in red spruce carbon balance and allocation in response to interacting ozone and nutrient stresses. *Tree Physiology* 9: 127-146.

Weinstein, D A and R Beloin. 1990. Evaluating effects of pollutants on integrated tree processes: A model of carbon, water, and nutrient balances. *Process Modeling of Forest Growth Responses to Environmental Stress* 313-323.

Weishampel, J F and D L Urban. 1996. Coupling a spatially-explicit forest gap model with a 3-D solar routine to simulate latitudinal effects. *Ecological Modelling* 86: 101-111.

White, P S. 1979. Pattern, process, and natural disturbance in vegetation. *The Botanical Review* 45: 229-299.

Whitmore, T C. 1989. Canopy gaps and the two major groups of forest trees. *Ecology* 70: 536-538.

With, K A. 2002. The landscape ecology of invasive spread. *Conservation Biology* 16: 1192-1203.

Wittig, V E, E A Ainsworth, S L Naidu, D F Karnosky, S P Long, and A R S USDA. 2009. Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: A quantitative meta-analysis. 15: 396-424.

Wittig, V E, E A Ainsworth, and S P Long. 2007. To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last 3 decades of experiments. *Plant Cell and Environment* 30: 1150-62.

Woods, K D. 1993. Effects of invasion by *Lonicera tatarica* L. On herbs and tree seedlings in four New England forests. *American Midland Naturalist* 130: 62-74.

Wyckoff, P H and S L Webb. 1996. Understory influence of the invasive Norway maple (*Acer platanoides*). *Bulletin of the Torrey Botanical Club* 123: 197-205.

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Table 1: Species rank for net photosynthesis rates two days after ozone trials (Elton Chapter 1), shade tolerance classification (1 very shade tolerant-5 very shade intolerant).

		Ozone regimes		Shade tolerance
Species	Low	Medium	High	
L. tulipifera	1	5	5	5
R. pseudoacacia	2	4	3	5
A. altissima	3	3	9	5
A. platanoides	4	7	7	1
Q. acutissima	5	12	2	2
P. calleryana	6	1	1	2
C. glabra	7	11	4	2
C. occidentalis	8	6	8	3
P. tomentosa	9	9	6	4
P. serotina	10	2	10	2
Q. rubra	11	10	12	1
Q. alba	12	13	13	1
A. rubrum	13	8	11	2

Table 2: Relative density of species from model validation compared to values found in previous studies on forest stands in Virginia.

Source	Stand location	Relative Density (%)	Species
Elton	Virginia	24	*Carya glabra*
		21	*Acer rubrum*
		15	*Quercus rubra*
		12	*Quercus alba*
		11	*Celtis occidentalis*
		9	*Prunus serotina*
		5	*Robinia pseudoacacia*
		3	*Liriodendron tulipifera*
McCormick and Platt 1980	Virginia	31	*Acer rubrum*
		17	*Carya glabra*
		7	*Quercus alba*
		7	*Quercus rubra*
Farrell and Ware 1991	Virginia	20	*Carya* spp.
		13	*Quercus alba*
		11	*Acer rubrum*
		9	*Liriodendron tulipifera*
		3	*Quercus rubra*

Table 3: Spearman rank correlation coefficients (r_s) for the relative abundance of each species across all plots. Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.

Time	O_3 regimes		r_s
		Medium	High
50	Low	0.55	0.99***
	High	0.73**	
100	Low	0.63*	0.81**
	High	0.47	
150	Low	0.62*	0.78*
	High	0.44	
200	Low	0.52	0.85**
	High	0.45	

Table 4: Pearson correlation coefficients for abundance, mean DBH, and mean height at 50, 100, 150, and 200 years of simulation for all species**. Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.**

Year		DBH	Height
50			
	Abundance	0.09***	0.42**
	DBH		0.93***
100			
	Abundance	0.29***	0.27***
	DBH		0.94***
150			
	Abundance	0.36***	0.32***
	DBH		0.94***
200			
	Abundance	0.40***	0.35***
	DBH		0.94***

Table 5: Multivariate analysis of variance pairwise comparisons testing the effect of ozone (fixed effect) on relative abundance, diameter at breast height (DBH), and height as dependent vectors for native and invasive species by year.

					Abundance	DBH	Height
		Source	ndf	ddf	*F*	*F*	*F*
Forest Age	Contrast	Ozone					
50							
	High-Low		2	98	21.76***	70.03***	66.95***
	Medium-High		2	98	83.19***	17.91***	19.86***
	Medium-Low		2	98	83.19***	84.84***	69.68***
100							
	High-Low		2	98	4.46*	12.19***	2.90
	Medium-High		2	98	141.90***	127.53***	101.25***
	Medium-Low		2	98	121.11***	71.64***	64.62***
150							

High-Low	2	98	5.70**	22.36***	21.30***
Medium-High	2	98	147.68***	175.98***	129.30***
Medium-Low	2	98	103.18***	53.18***	33.73***

200

High-Low	2	98	19.69***	33.96***	34.77***
Medium-High	2	98	166.91***	156.17***	146.40***
Medium-Low	2	98	190.88***	49.98***	31.92***

Table 6: Multivariate analysis of variance pairwise comparisons ran for all 13 species by forest age. Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.

					Abundance	DBH	Height
Forest Age	Contrast	Source Ozone	ndf	ddf	*F*	*F*	*F*
50	High-Low		13	87	18.43***	81.31***	69.16***
	Medium-High		13	87	69.57***	145.29***	96.50***
	Medium-Low		13	87	48.76***	108.23***	75.58***
100	High-Low		13	87	26.46***	37.86***	31.13***
	Medium-High		13	87	161.20***	128.01***	88.79***
	Medium-Low		13	87	102.56***	75.50***	55.11***
150	High-Low		13	87	29.37***	48.63***	36.86***

	Medium-High	13	87	131.73***	157.94***	108.73***
	Medium-Low	13	87	92.15***	84.39***	62.84***
200	High-Low	13	87	35.33***	54.08***	42.33***
	Medium-High	13	87	146.14***	189.23***	134.01***
	Medium-Low	13	87	128.45***	116.23***	91.76***

Table 7: Multivariate analysis of variance for the effect of ozone on relative abundance, DBH, and height of each simulated species by forest age. Significance indicated by: *p<0.05, **p<0.001, ***p<0.0001.

				Abundance	DBH	Height
Source		ndf	ddf	*F*	*F*	*F*
Ozone	Forest Age					
	50	39	844.69	32.74**	67.37***	50.35***
	100	39	844.69	10.88***	49.20***	39.12***
	150	39	844.69	47.65***	45.19***	37.25***
	200	39	844.69	52.31***	51.59***	45.68***

Figure Legend:

Figure 1: a. The modeled abundance (trees/hectare) for native species in the model validation simulations. b. The modeled biomass (metric ton/hectare) for native species in the model validation simulations. Species included are *Robinia pseudoacacia* (ROPS), *Quercus rubra* (QURU), *Quercus alba* (QUAL), *Prunus serotina* (PRSE), *Liriodendron tulipifera* (LITU), *Celtis occidentalis* (CEOC), *Carya glabra* (CAGL), and *Acer rubrum* (ACRU).

Figure 2: The modeled abundance (trees/hectare) for species in the low ozone forest for 200 years. Species included are *Robinia pseudoacacia* (ROPS), *Quercus rubra* (QURU), *Quercus alba* (QUAL), *Prunus serotina* (PRSE), *Liriodendron tulipifera* (LITU), *Celtis occidentalis* (CEOC), *Carya glabra* (CAGL), *Acer rubrum* (ACRU), *Pyrus calleryana* (PYCA), *Paulownia tomentosa* (PATO), *Quercus acutissima* (QUAC), *Ailanthus altissima* (AIAL), and *Acer platanoides* (ACPL).

Figure 3: The modeled abundance (trees/hectare) for species in the medium ozone forest for 200 years. Species included are *Robinia pseudoacacia* (ROPS), *Quercus rubra* (QURU), *Quercus alba* (QUAL), *Prunus serotina* (PRSE), *Liriodendron tulipifera* (LITU), *Celtis occidentalis* (CEOC), *Carya glabra* (CAGL), *Acer rubrum* (ACRU), *Pyrus calleryana* (PYCA), *Paulownia tomentosa* (PATO), *Quercus acutissima* (QUAC), *Ailanthus altissima* (AIAL), and *Acer platanoides* (ACPL).

Figure 4: The modeled abundance (trees/hectare) for species in the high ozone forest for 200 years. Species included are *Robinia pseudoacacia* (ROPS), *Quercus rubra* (QURU), *Quercus alba* (QUAL), *Prunus serotina* (PRSE), *Liriodendron tulipifera* (LITU), *Celtis occidentalis* (CEOC), *Carya glabra* (CAGL), *Acer rubrum* (ACRU), *Pyrus calleryana* (PYCA), *Paulownia tomentosa* (PATO), *Quercus acutissima* (QUAC), *Ailanthus altissima* (AIAL), and *Acer platanoides* (ACPL).

Figure 5: The species diversity (Shannon diversity index) for the low, medium, and high ozone regimes at 50, 100, 150, 200 years.

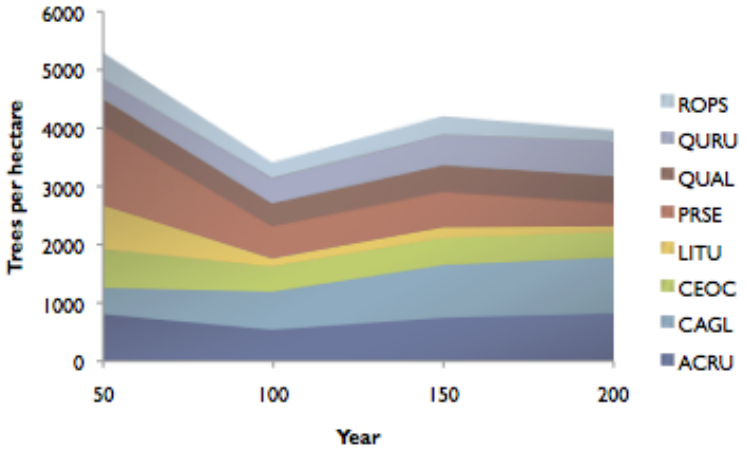
Figure 6: Frequency distribution of diameter at breast-height (DBH) classes after 200 years for low, medium and high ozone simulation for a) *Quercus alba* b) *Quercus rubra* c) *Carya glabra* d) *Liriodendron tulipifera* e) Acer *rubrum* f) *Celtis occidentalis* g) *Prunus serotina* h) *Robinia pseudoacacia* i) *Quercus acutissima* j) *Ailanthus altissima* k) *Acer platanoides* l) *Paulownia tomentosa*, and m) *Pyrus calleryana*. The number of trees per hectare (n), and the mean diameter at base height (xbar) are indicated on each graph.

Figure 7: Canopy composition of a) low, b) medium, and c) high ozone forest. The height of each tree represents the average canopy height (meters) and the width of the crown represents the percent of the canopy occupied. Each tree represents one of the following species: *Ailanthus altissima* (yellow), *Acer rubrum* (red), *Carya glabra* (blue), and *Prunus serotina* (green).

Figure 8: Frequency distribution of diameter at breast-height (DBH) classes for low, medium, and high ozone simulation at a) 50, b) 100, c) 150, and d) 200 years. The number of trees per hectare (n), and the mean diameter at base height (xbar) are indicated on each graph. All multivariate analysis of variance pairwise comparisons resulted in significant differences where $p<0.0001$.

Figure 1:

a.


6000
5000
4000
3000
2000
1000
0
Trees per hectare
50
100
150
200
Year
ROPS
QURU
QUAL
PRSE
LITU
CEOC
CAGL
ACRU

b.

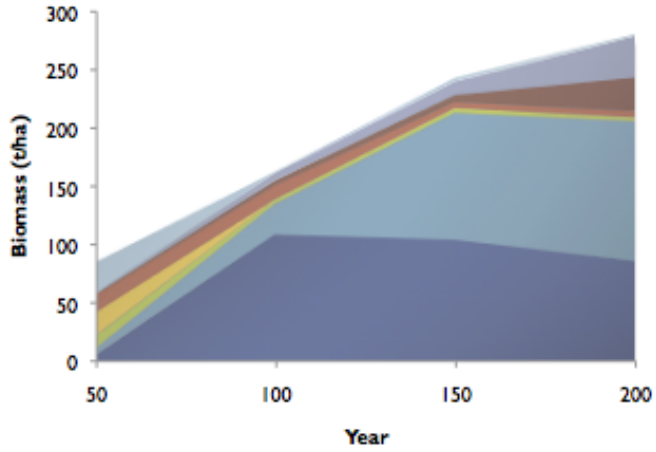

300
250
200
150
100
50
0
Biomass (t/ha)
50
100
150
200
Year

Figure 2:

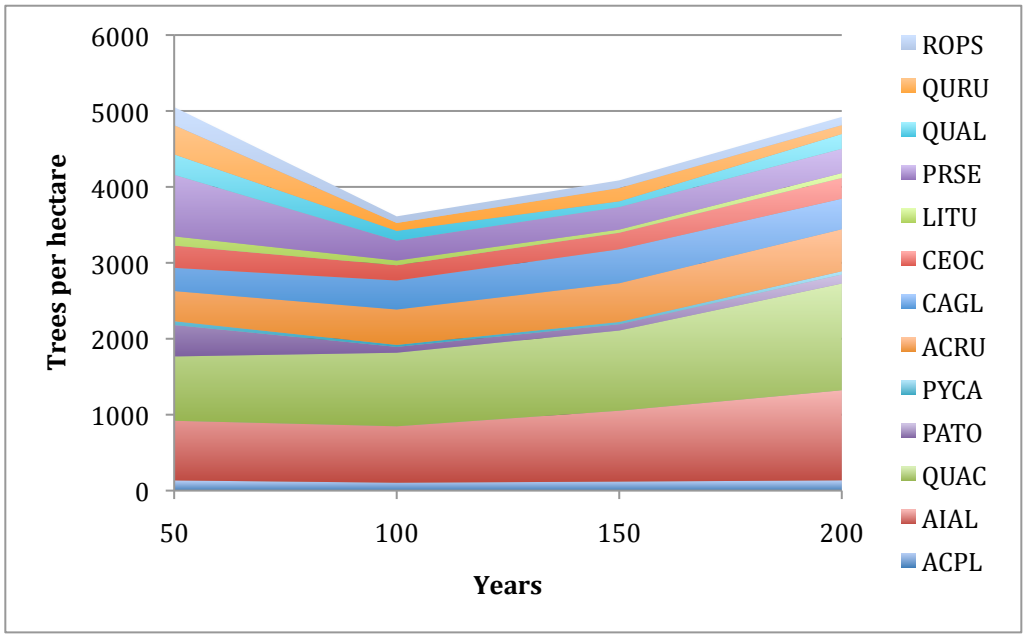

6000
5000
4000
3000
2000
1000
0
Trees per hectare
50
100
150
200
Years
ROPS
QURU
QUAL
PRSE
LITU
CEOC
CAGL
ACRU
PYCA
PATO
QUAC
AIAL
ACPL

Figure 3:

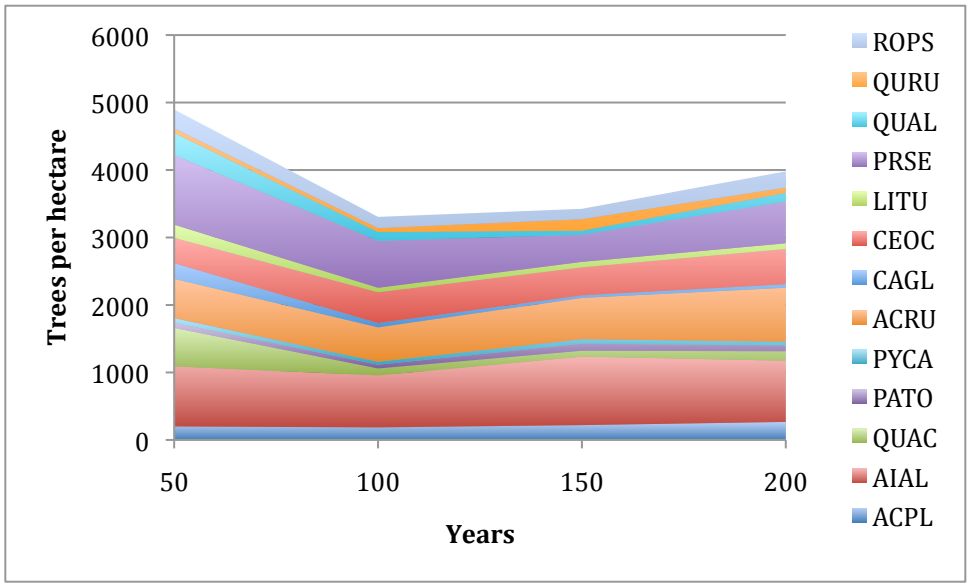

6000
5000
4000
3000
2000
1000
0
Trees per hectare
50
100
150
200
Years
ROPS
QURU
QUAL
PRSE
LITU
CEOC
CAGL
ACRU
PYCA
PATO
QUAC
AIAL
ACPL

Figure 4:

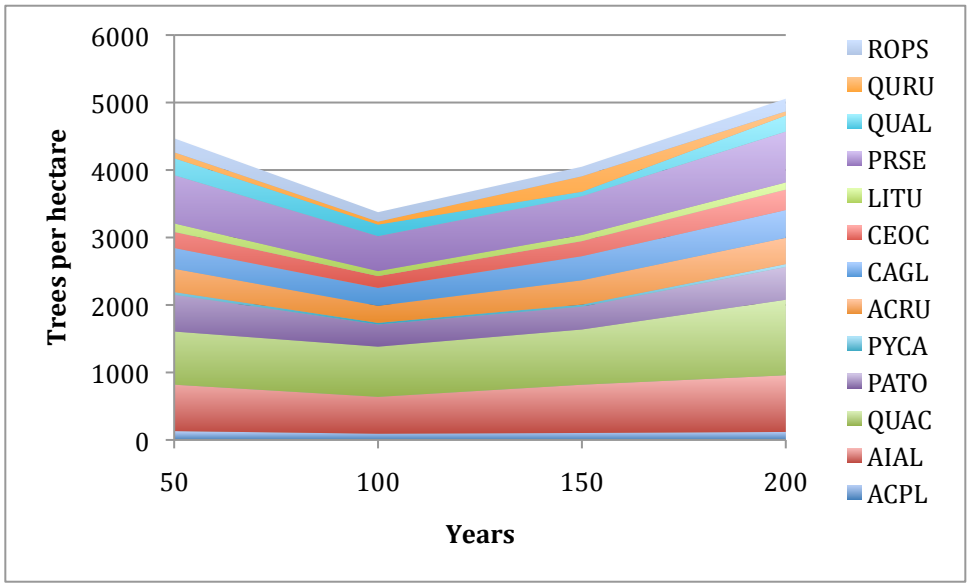

6000
5000
4000
3000
2000
1000
0
Trees per hectare
50
100
150
200
Years
ROPS
QURU
QUAL
PRSE
LITU
CEOC
CAGL
ACRU
PYCA
PATO
QUAC
AIAL
ACPL

Figure 5:

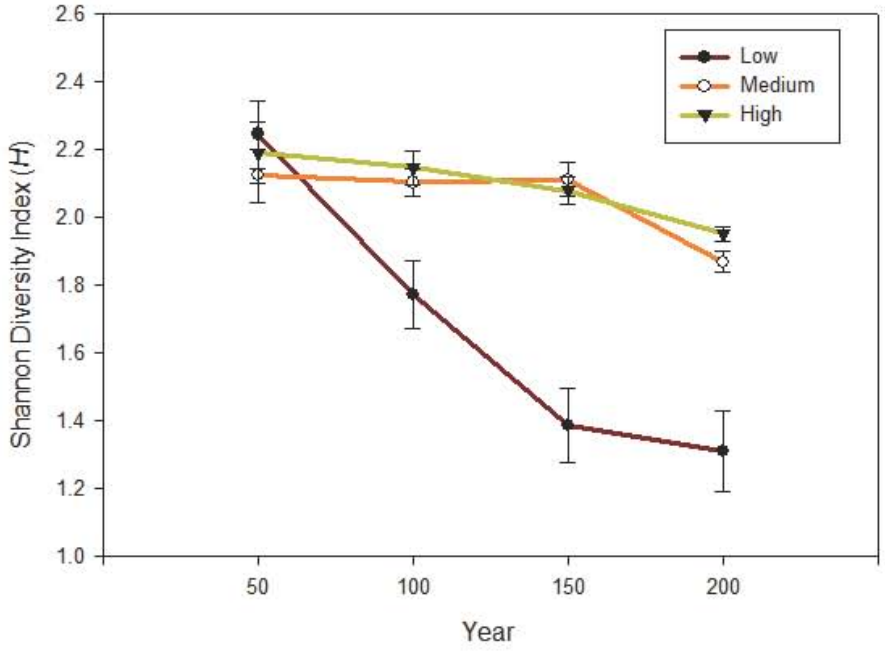
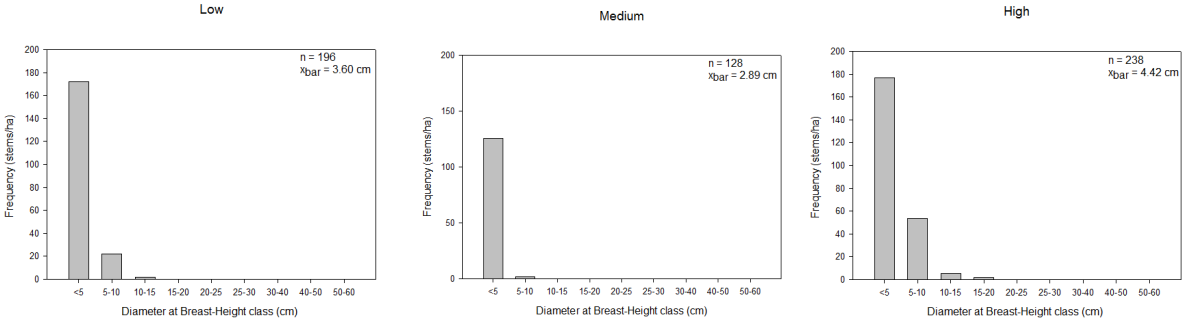
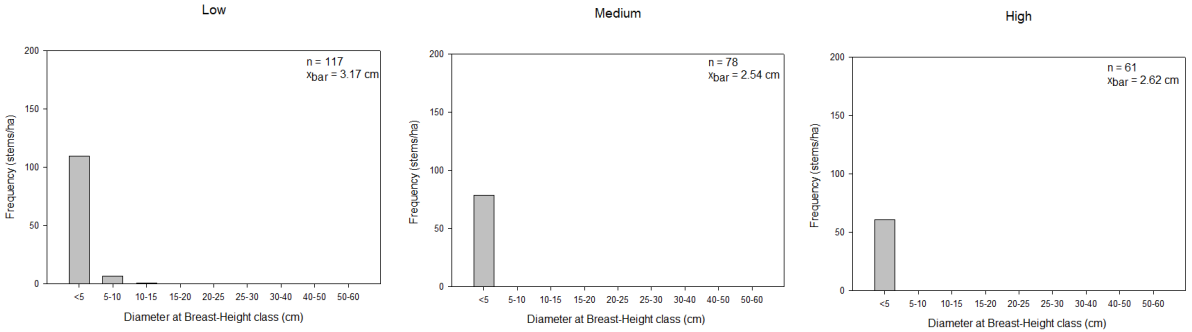

Low
Medium
High
Shannon Diversity Index (H)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
50
100
150
200
Year

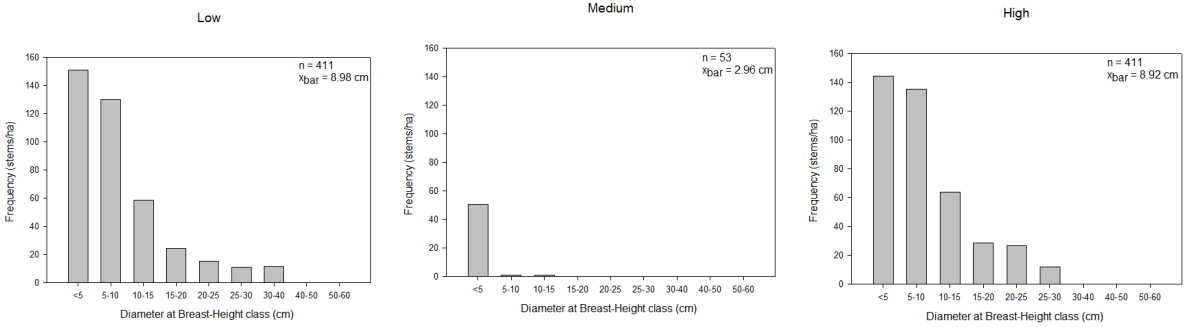
Figure 6a: *Quercus alba*



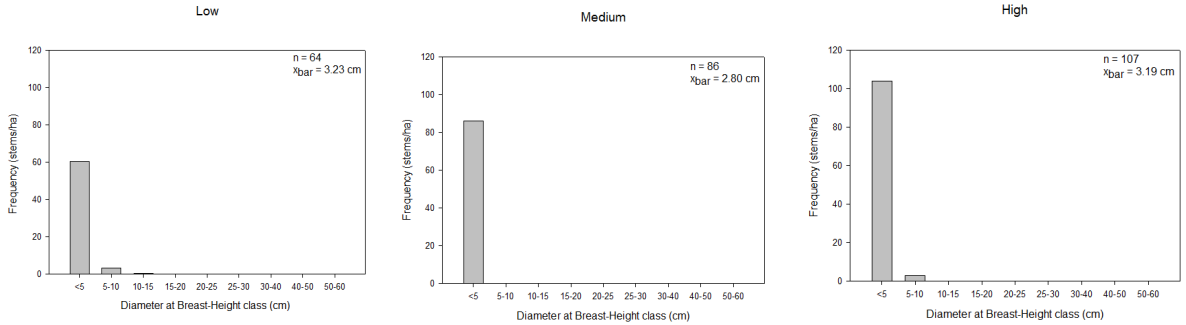
b: *Quercus rubra*



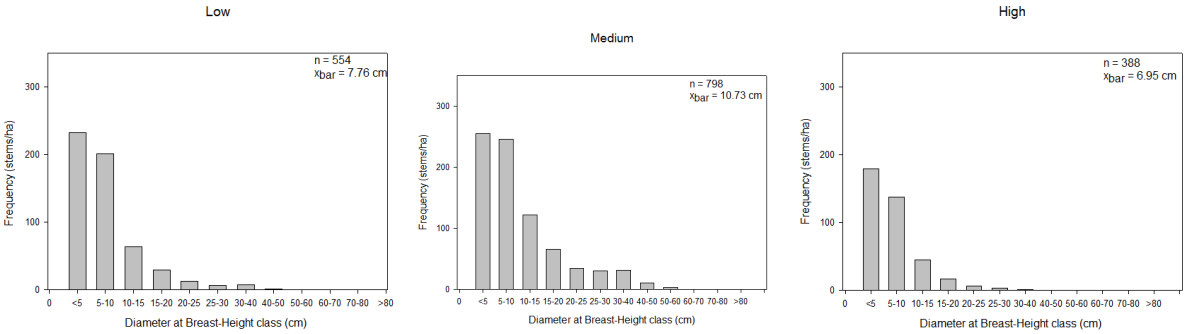
c: *Carya glabra*



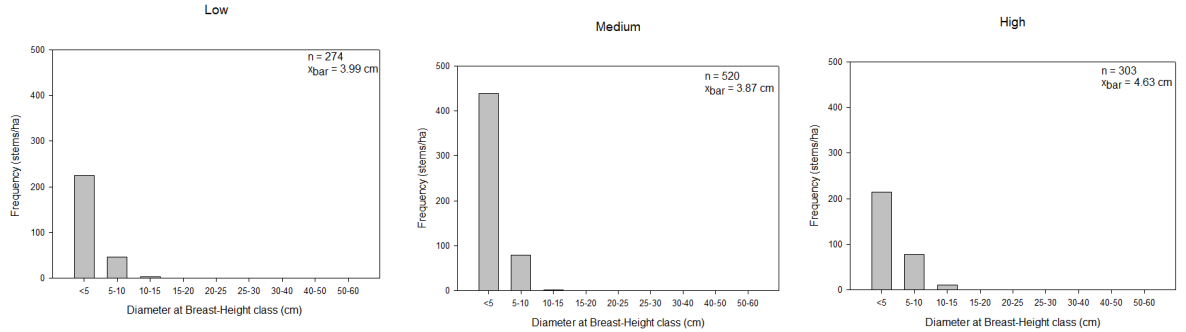
d: ***Liriodendron tulipifera***



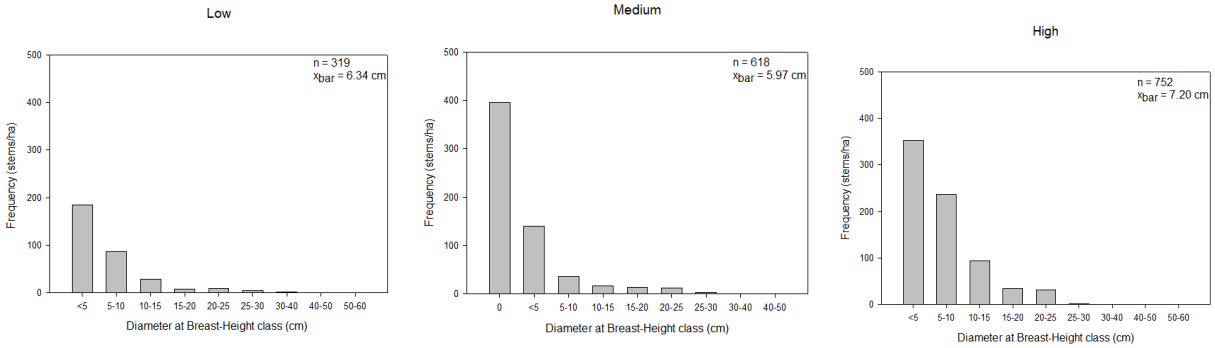
e: ***Acer rubrum***



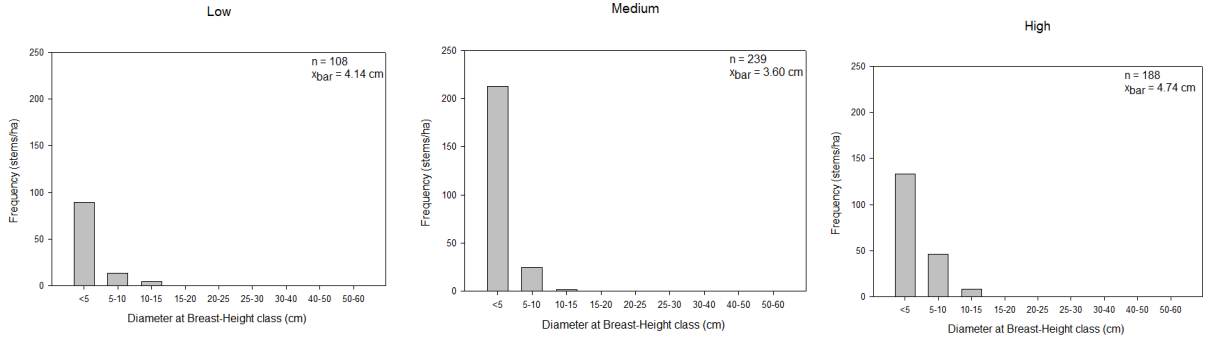
f: ***Celtis occidentalis***



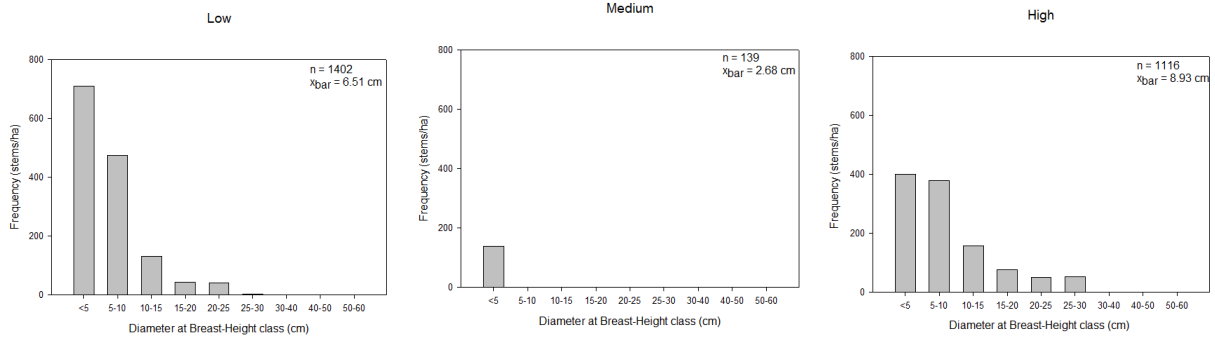
g: *Prunus serotina*



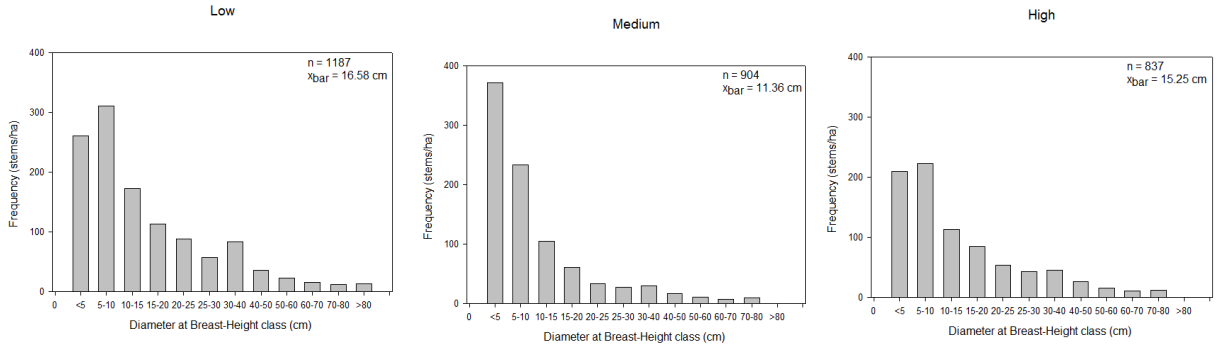
h: *Robinia pseudoacacia*



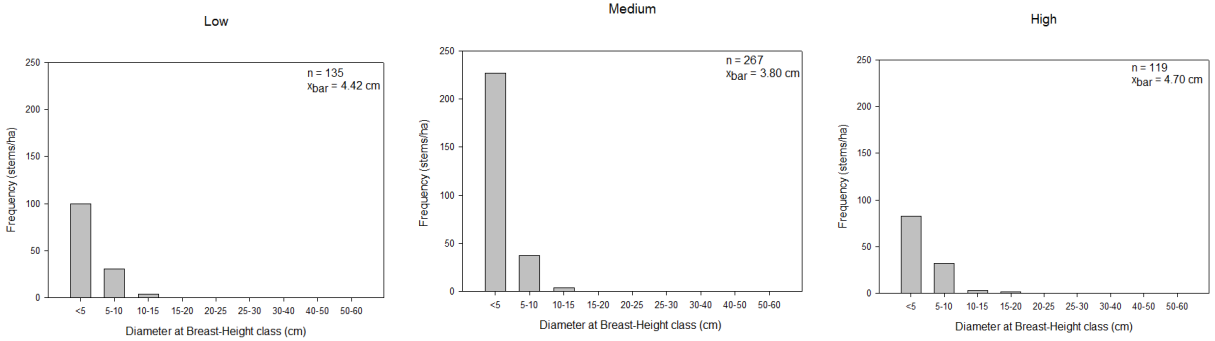
i: *Quercus acutissima*



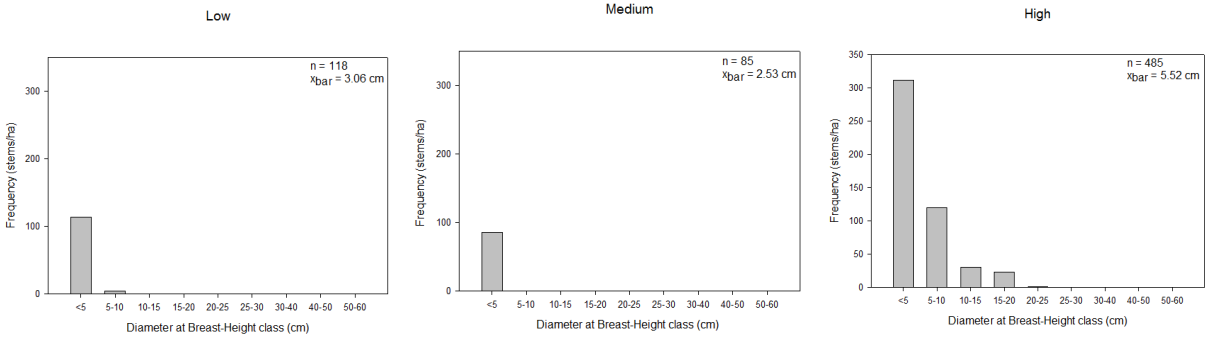
j: ***Ailanthus altissima***



k: ***Acer platanoides***



l: ***Paulownia tomentosa***



m: ***Pyrus calleryana***

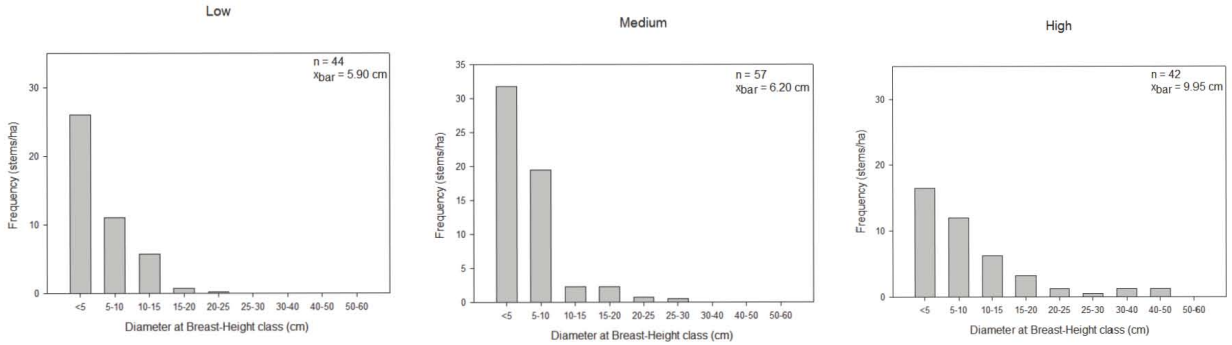
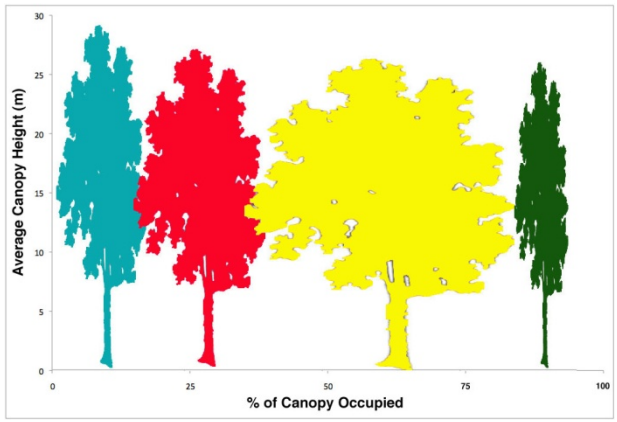
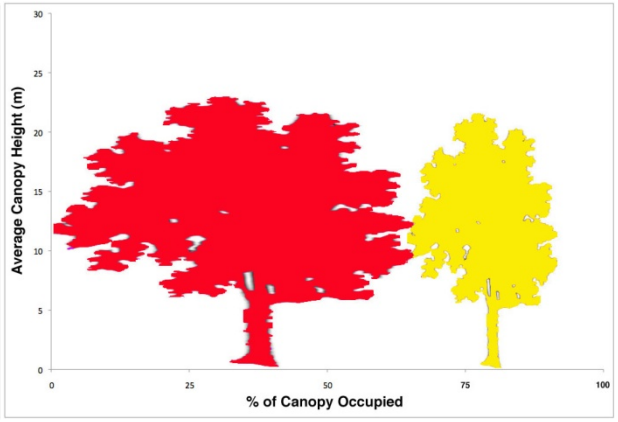


Figure 7:

a:


Average Canopy Height (m)
% of Canopy Occupied
0
5
10
15
20
25
30
0
25
50
75
100

b:


Average Canopy Height (m)
% of Canopy Occupied
0
5
10
15
20
25
30
0
25
50
75
100

c:

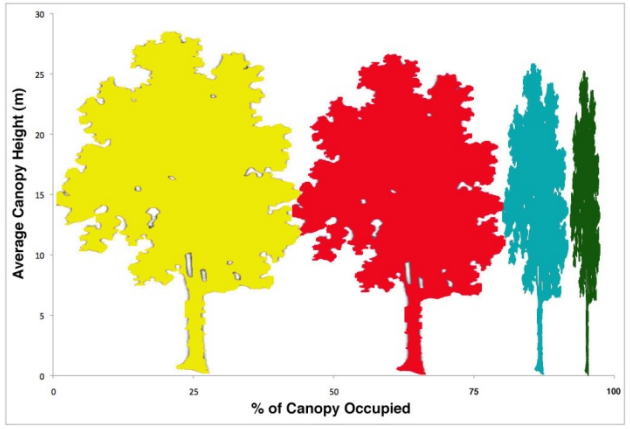
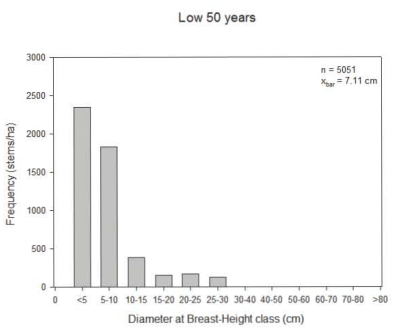
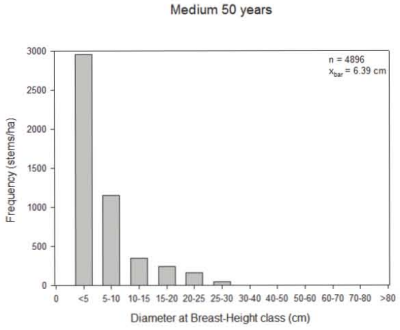
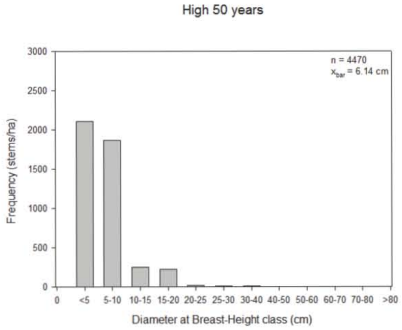

Average Canopy Height (m)
% of Canopy Occupied
0
5
10
15
20
25
30
0
25
50
75
100

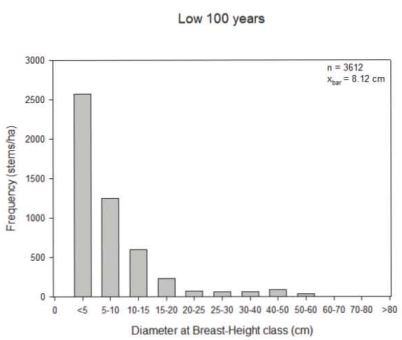
Figure 8a:

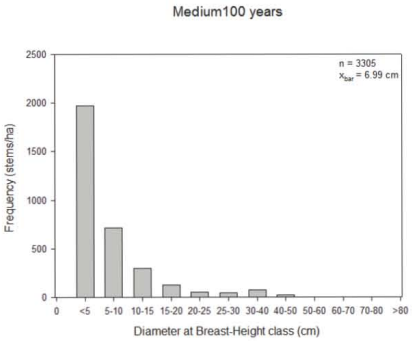

Low 50 years
n = 5051
x̄bar = 7.11 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)

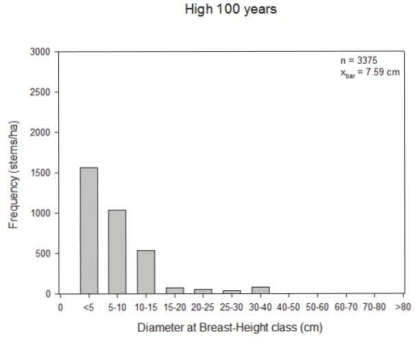

Medium 50 years
n = 4896
x̄bar = 6.39 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)


High 50 years
n = 4470
x̄bar = 6.14 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)

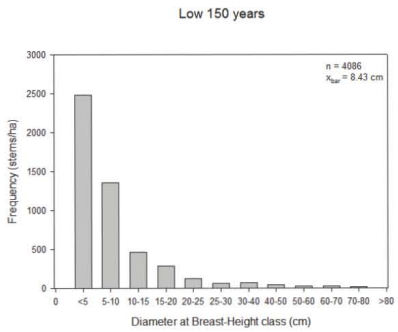
b:

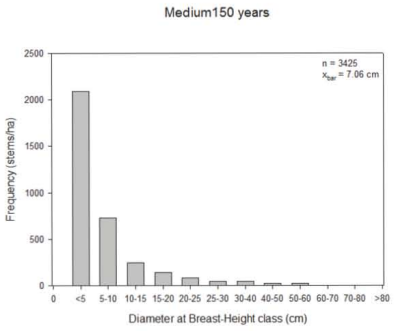

Low 100 years
n = 3612
x̄bar = 8.12 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)

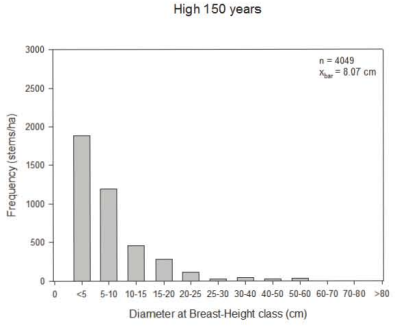

Medium100 years
n = 3305
x̄bar = 6.99 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)


High 100 years
n = 3375
x̄bar = 7.59 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)

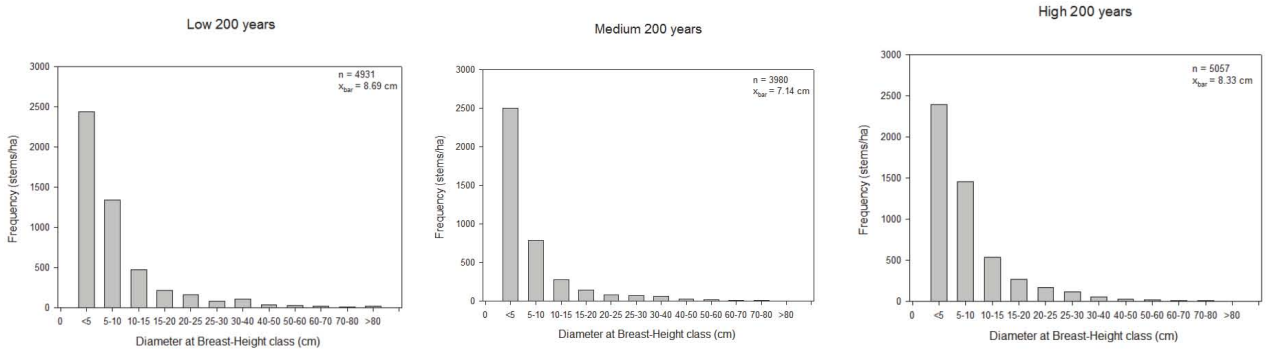
c:


Low 150 years
n = 4086
x̄bar = 8.43 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)


Medium150 years
n = 3425
x̄bar = 7.06 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)


High 150 years
n = 4049
x̄bar = 8.07 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)

d:


Low 200 years
n = 4931
x_{bar} = 8.69 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)
Medium 200 years
n = 3980
x_{bar} = 7.14 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)
High 200 years
n = 5057
x_{bar} = 8.33 cm
Frequency (stems/ha)
Diameter at Breast-Height class (cm)

Appendix I: Sources used for biomass estimations by species.

Tree Species	Leaf Biomass	Branch Structure	Branch TNC	Branch Wood	Stem Structure	Stem TNC	Stem Wood	Coarse Root	Fine Root
Acer rubrum	Martin et al. 1998	Kinerson and Bartholomew 1977	Crow and Erdmann 1983	Crow and Erdmann 1983	Kinerson and Bartholomew 1977	Ribe 1973	Ker 1984	Ker 1985	Young et al. 1980
Acer platanoides	Withington et al. 2006	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Withington et al. 2006	Withington et al. 2006
Ailanthus altissima	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Clark and Schroeder 1986	Clark and Schroeder 1986

	Martin et al.	Hitchcock	Hitchcock	Hitchcock	Phillips	Phillips	Phillips	Clark and Schroeder	Clark and Schroeder
Carya glabra	1998	1978	1978	1978	1977	1977	1977	1986	1986
Celtis occidentalis	Shipley 2002	Kasile 1984	Kasile 1985	Kasile 1986	Kasile 1987	Kasile 1988	Kasile 1989	Raile 1982	Raile 1982
Liriodendron tulipifera	Norby and O'Neill 1991	Vogt 1991	Jensen and Patton 1990	Panshin and de Zeeuw 1980	Vogt 1991	Jensen and Patton 1990	Panshin and de Zeeuw 1980	Vogt 1991	Panshin and de Zeeuw 1980
Paulownia tomentosa	Nowak 1996	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Frieswyk and DiGiovanni 1989	Clark and Schroeder 1986	Clark and Schroeder 1986
Prunus serotina	Klooster et al. 2007	DeLucia et al.1998	DeLucia et al. 1998	DeLucia et al. 1998	DeLucia et al. 1998	DeLucia et al. 1998	DeLucia et al. 1998	DeLucia et al. 1998	DeLucia et al. 1998

	Peper	Clark and	Clark and	Clark and	Clark and	Clark and	Clark and	Clark and	Clark and
Pyrus	et al.	Schroeder	Schroeder	Schroeder	Schroeder	Schroeder	Schroeder	Schroeder	Schroeder
calleryana	2001	1986	1986	1986	1986	1986	1986	1986	1986
Quercus	Bridge	Bridge	Bridge	Bridge	Bridge	Bridge	Bridge	Bridge	Bridge
acutissima	1979	1979	1980	1981	1982	1983	1984	1985	1991
	Martin								
	et al.	Martin et	Martin et	Martin et	Martin et	Martin et	Martin et	Comas et	Comas et
Quercus alba	1998	al. 1998	al. 1999	al. 2000	al. 2001	al. 2002	al. 2003	al. 2002	al. 2002
		Goldsmith	Goldsmith	Goldsmith		Goldsmith	Goldsmith		
	Martin	and	and	and	Goldsmith	and	and		
Quercus	et al.	Hocker	Hocker	Hocker	and Hocker	Hocker	Hocker	Comas et	Comas et
rubra	1998	1978	1978	1979	1980	1981	1982	al. 2002	al. 2002
	Mitchel								
Robinia	l et al.	Clark et al.	Clark et al.	Clark et al.	Clark et al.	Clark et al.	Clark et al.	Clark et	Clark et
pseudoacacia	1999	1985	1985	1986	1987	1988	1989	al. 1990	al. 1996

CHAPTER 4

Conclusion

The findings presented in this study provide evidence that different broadleaf deciduous tree species show a wide range of responses to ground-level O_3. At O_3 concentrations common during high O_3 events in the Mid-Atlantic region, some species performed poorly while others maintained high net photosynthesis. This variety of ozone susceptibilities demonstrates the potential of O_3 to harm certain species thereby causing shifts in competitive interactions among species. If the competitive ability of a species was altered, this could lead to eventual changes in forest composition.

The species-specific responses noted during and after the gas-exchange experiments were only partially explained by ascorbic acid concentrations in the leaves. The relationship between post-exposure ascorbic acid and the ozone tolerance was well explained for the two species with highest post-exposure ascorbic acid (*Prunus serotina*, *Acer rubrum*), but all other species had decreases in ASA. Moreover, the three species that had ASA levels below the level of reliable quantification were not among the most ozone sensitive species. For example, net photosynthesis in *Pyrus calleryana* actually increased with increasing concentrations of ozone despite having leaves with ASA below the quantifiable level. Therefore, the mechanisms for coping with ozone stress may be varied and species specific. Some species (*P. serotina* and *A. rubrum*) may rely heavily on ASA pathways for oxidant detoxification. Other species (*P. calleryana*) might rely on non-ASA pathways to detoxify oxidative damage.

These physiological differences could translate into changes in community composition and structure under elevated O_3 conditions. The sharp reduction in net photosynthesis in some species indicated the potential for decreases in competitive ability

relative to the more O_3 tolerant species. Such competitive shifts at elevated O_3 levels were confirmed in model simulations based on physiological O_3 tolerance. Large changes in net photosynthesis measured in the first chapter did not always result in large changes in competitive ability. The large photosynthetic changes did not always result in large competitive shifts due to variations in light interception, ambient temperature, and precipitation, which affect tree growth within the model.

Many of the pioneer species, those shade intolerant natives and invasive species, greatly benefitted from establishing on a bare field, and the shifts in competition would have been much more subtle if the simulations began within an established forest. As the forest matured, the shifts in competition due to ozone sensitivity increased as light became limiting. After canopy closure, only the shade tolerant ozone tolerant species benefited from their ozone tolerance.

Native and invasive species responded similarly to ozone treatments during the gas-exchange trails, but invasive species decreased ASA after exposures while native species tended to increase ASA. This resulted in the two species groups having comparable post-exposure ASA, while invasive species had significantly higher constitutive ASA. Though native and invasive species did not respond differently to O_3, it was hypothesized that the inclusion of competition would cause invasive species to outperform native species. However, the community patterns seen when looking at native and invasive species composition demonstrated that even with competition native and invasive species did not differ in response to ozone concentrations. Invasive species were in higher relative abundance than native species in the low and medium ozone environment, mostly due to the success of *Ailanthus altissima* in the overstory and *Quercus acutissima* in the

understory. Native species dominated in the medium ozone environment due to the success of *A. rubrum* outcompeting *A. altissima* early in the simulation due to increases in ozone sensitivity.

The ability to quantify the O_3 responses of a large number of tree species provided this study with greater strength than previous studies in assessing the effect of ozone on Mid-Atlantic forest communities. The competition for light within the gap model did not allow for as wide of a range of response as seen with net photosynthesis in the gas-exchange experiments, when light was not limiting. The competition for light in forest systems exacerbates the stress caused by ozone, but also prevents ozone sensitivity from acting as a dominant restructuring characteristic. As trees are predominantly limited by light in forest systems, O3 stress affects trees to a lesser extent than competition for light.

Dissertation Appendix I: Urban forest surveys performed during summer 2006 including a low, medium, and high ozone level site around three urban metropolises (Washington, DC; Baltimore, MA; Philadelphia, PA). Survey methods followed USDA Forest Service ozone biomonitoring standards, where four 100 m transects were made from a central point. Each species greater than or equal to 1 cm in DBH was included.

Site									
Ozone level									
Coordinates									
Lindwood, PA					**Density**	**Mean**		**Ozone**	**Ozone**
Low		**Species**	**Status**	**Count**	**ha^{-1}**	**DBH**	**IV**	**Damage**	**IV**
N39.92	W-75.53	*Acer platanoides*	invasive	1	32.40	21.00	7.02	0	0.00
		Acer rubrum	native	5	162.00	49.40	25.05	0	0.00
		Ailanthus altissima	invasive	1	32.40	18.00	6.80	0	0.00
		Alliaria petiolata	invasive	1	32.40	1.00	5.52	0	0.00
		Asimina triloba	native	1	32.40	13.00	6.42	0	0.00
		Campsis radicans	native	1	32.40	1.00	5.52	0	0.00
		Carya cordiformis	native	1	32.40	37.00	8.23	0	0.00

	Catulpa speciosa	native	1	32.40	34.00	8.01	0	0.00
	Celtis occidentalis	native	3	97.20	64.00	21.15	0	0.00
	Cercis canadenis	native	1	32.40	21.00	7.02	0	0.00
	Cornus florida	native	1	32.40	22.00	7.10	0	0.00
	Fraxinus pennsylvanica	native	1	32.40	41.00	8.53	1	2.84
	Hamamelis virginiana	native	1	32.40	25.00	7.33	0	0.00
	Hydrangea arborescens	native	1	32.40	8.00	6.04	0	0.00
	Juglans nigra	native	1	32.40	23.00	7.18	0	0.00
	Liriodendron tulipifera	native	4	129.60	29.00	15.13	1	1.26
	Lonicera japonica	invasive	1	32.40	1.00	5.52	0	0.00
	Parthenocissus quinquefolia	native	2	64.80	1.50	11.00	0	0.00
	Pinus strobus	native	1	32.40	63.00	10.19	0	0.00
	Platanus occidentalis	native	1	32.40	181.0 0	19.09	0	0.00
	Prunus serotina	native	1	32.40	28.00	7.55	0	0.00
	Quercus marilandica	native	1	32.40	48.00	9.06	0	0.00

		Quercus rubra	native	2	64.80	55.50	15.07	0	0.00
		Rhus typhina	native	1	32.40	11.00	6.27	0	0.00
		Robinia pseudoacacia	native	1	32.40	54.00	9.51	0	0.00
		Rubus odoratus	native	4	129.60	1.50	18.94	0	0.00
Pulaski Park, PA									
Medium									
N40.14	W-75.30	*Acer negundo*	native	1	47.36	45.00	9.87	0	0.00
		Acer platanoides	invasive	1	47.36	52.00	10.55	0	0.00
		Acer rubrum	native	1	47.36	18.00	7.21	1	2.40
		Acer saccharinum	native	1	47.36	84.00	13.70	0	0.00
		Ailanthus altissima	invasive	1	47.36	23.00	7.70	0	0.00
		Broussonetia papyrifera	native	2	94.72	18.00	12.65	2	4.22
		Cornus florida	native	1	47.36	25.00	7.90	0	0.00
		Juglans nigra	native	1	47.36	87.00	14.00	1	4.67
		Lespedeza cuneata	invasive	1	47.36	1.00	5.54	0	0.00
		Liquidambar styraciflua	native	1	47.36	58.00	11.14	0	0.00

	Lonicera maackii	invasive	1	47.36	8.00	6.23	0	0.00
	Morus alba	native	3	142.09	30.33	19.31	0	0.00
	Parthenocissus quinquefolia	native	1	47.36	1.00	5.54	2	3.69
	Paulownia tomentosa	invasive	2	94.72	26.50	13.49	0	0.00
	Populus abla	invasive	1	47.36	95.00	14.78	1	4.93
	Quercus acutissima	invasive	1	47.36	26.00	8.00	0	0.00
	Quercus alba	native	1	47.36	43.00	9.67	3	9.67
	Quercus rubra	native	1	47.36	31.00	8.49	0	0.00
	Rhus typhina	native	5	236.81	16.00	19.96	0	0.00
	Robina pseudoacacia	native	5	236.81	19.00	23.19	2	3.09
	Rosa multiflora	invasive	1	47.36	2.00	5.64	0	0.00
	Rubus odoratus	native	5	236.81	2.60	24.52	3	4.90
	Salix babylonica	invasive	1	47.36	43.00	9.67	2	6.45
	Vitis labrusca	native	1	47.36	7.00	6.13	1	2.04
Fairmont Park, PA								
High								

N39.99	W-75.21	*Acer platanoides*	invasive	9	396.80	43.00	41.43	1	1.53
		Acer rubrum	native	1	44.09	15.00	6.91	2	4.61
		Ailanthus altissima	invasive	2	88.18	31.50	13.97	0	0.00
		Alliaria petiolata	invasive	2	88.18	1.50	11.03	0	0.00
		Broussonetia papyrifera	native	2	88.18	22.50	13.09	4	8.73
		Celastrus orbiculatus	native	3	132.27	4.67	16.78	0	0.00
		Cornus florida	native	1	44.09	29.00	8.29	0	0.00
		Fraxinus americana	native	1	44.09	42.00	9.56	2	6.38
		Gymnocladus dioicus	native	1	44.09	15.00	6.91	2	4.61
		Hedra helix	invasive	2	88.18	1.50	11.03	0	0.00
		Liriodendron tulipifera	native	2	88.18	39.00	14.71	7	17.16
		Lonicera japonica	invasive	1	44.09	1.00	5.54	0	0.00
		Lonicera maackii	invasive	1	44.09	5.00	5.93	0	0.00
		Parthenocissus quinquefolia	native	2	88.18	2.00	11.08	3	5.54
		Paulownia tomentosa	invasive	2	88.18	48.50	15.64	0	0.00
		Phytolacca americana	native	1	44.09	12.00	6.62	0	0.00

		Prunus serotina	native	3	132.27	32.67	16.59	8	14.74
		Robinia pseudoacacia	native	2	88.18	50.50	15.84	0	0.00
		Vitis labrusca	native	2	88.18	3.50	8.28	6	8.28
Patapsco Valley Park, MD									
Low									
N39.83	W-77.98	*Acer pensylvanicum*	native	2	0.13	3.75	255.00	0	0.00
		Acer rubrum	native	12	1.50	3.54	871.67	1	1.56
		Aesculus octandra	native	1	0.06	5.00	302.50	0	0.00
		Ailanthus altissima	invasive	1	0.06	12.50	602.50	0	0.00
		Celastrus orbiculatus	invasive	3	0.19	2.50	307.50	0	0.00
		Dioscorea oppositifolia	invasive	1	0.06	12.50	602.50	0	0.00
		Fraxinus pennsylvanica	native	1	0.06	7.50	402.50	0	0.00
		Liriodendron tulipifera	native	2	0.25	3.75	355.00	1	2.63
		Parthenocissus quinquefolia	native	1	0.06	2.50	202.50	0	0.00
		Polygonum perfoliatum	invasive	1	0.06	12.50	602.50	0	0.00
		Prunus serotina	native	5	0.63	2.50	412.50	2	3.20

		Quercus alba	native	1	0.06	5.00	302.50	2	5.20
		Quercus marilandica	native	2	0.13	12.50	705.00	0	0.00
		Quercus nigra	native	1	0.13	2.50	202.50	2	10.00
		Quercus rubra	native	2	0.13	3.75	355.00	2	5.28
		Rhus typhina	native	2	0.13	2.50	205.00	0	0.00
		Rubus odoratus	native	1	0.13	2.50	202.50	0	0.00
		Vitis labrusca	native	1	0.06	2.50	202.50	1	2.03
Druid Hill Park, MD									
Medium									
N39.96	W-77.88	*Ailanthus altissima*	invasive	7	0.44	3.57	410.36	0	0.00
		Celastrus orbiculatus	invasive	2	0.13	3.75	255.00	0	0.00
		Fraxinus pennsylvanica	native	1	0.06	2.50	152.50	0	0.00
		Juglans nigra	native	1	0.06	5.00	252.50	5	17.54
		Kalmia latifolia	native	1	0.06	2.50	152.50	1	2.19
		Lonicera japonica	invasive	1	0.06	2.50	152.50	0	0.00
		Morus alba	native	2	0.13	6.25	355.00	0	0.00

		Parthenocissus quinquefolia	native	2	0.13	7.50	405.00	8	15.03
		Paulownia tomentosa	invasive	6	0.75	5.42	431.67	3	5.01
		Platanus occidentalis	native	2	0.25	12.50	555.00	0	0.00
		Prunus serotina	native	4	0.50	3.75	260.00	19	27.22
		Quercus alba	native	1	0.06	2.50	152.50	4	8.08
		Quercus marilandica	native	4	0.50	10.00	660.00	4	9.93
		Quercus rubra	native	1	0.06	2.50	152.50	4	10.99
		Rubus odoratus	native	3	0.38	7.50	457.50	0	0.00
		Vitis labrusca	native	2	0.13	3.75	255.00	1	2.00
Gunpowder Falls Park, MD									
High									
N40.12	W-77.57	*Acer negundo*	native	5	0.31	6.00	652.50	1	1.97
		Acer rubrum	native	1	0.06	2.50	202.50	4	13.51
		Ailanthus altissima	invasive	2	0.13	3.75	355.00	0	0.00
		Alliaria petiolata	invasive	3	0.19	5.00	507.50	0	0.00

		Dioscorea oppositifolia	invasive	1	0.06	5.00	302.50	0	0.00
		Fraxinus pennsylvanica	native	1	0.06	30.00	1302.5	3	12.71
		Juglans nigra	native	2	0.13	5.00	405.00	3	9.57
		Lonicera japonica	invasive	2	0.13	2.50	305.00	0	0.00
		Morus alba	native	1	0.06	2.50	202.50	1	4.21
		Platanus occidentalis	native	2	0.13	3.75	355.00	2	6.43
		Pueraria montana	invasive	2	0.13	3.75	255.00	0	0.00
		Pyrs americana	native	1	0.06	7.50	402.50	0	0.00
		Rhus typhina	native	1	0.06	5.00	302.50	0	0.00
		Rosa multiflora	invasive	13	0.81	4.81	924.81	5	7.07
		Vitis labrusca	native	3	0.19	8.33	640.83	6	11.73
Washington, DC									
Low									
N38.92	W-77.67	*Acer negundo*	native	1	0.06	5.00	302.50	0	0.00
		Amelanchier arborea	native	1	0.06	2.50	202.50	0	0.00
		Asimina triloba	native	2	0.13	17.50	905.00	0	0.00

	Carpinus caroliniana	native	1	0.06	7.50	402.50	0	0.00
	Celtis occidentalis	native	3	0.19	3.33	340.83	1	1.78
	Cornus florida	native	1	0.06	5.00	302.50	0	0.00
	Euonymus americanus	native	3	0.19	4.17	374.17	0	0.00
	Fraxinus pennsylvanica	native	4	0.25	6.88	585.00	1	1.90
	Kalmia latifolia	native	1	0.06	2.50	202.50	0	0.00
	Lonicera japonica	invasive	1	0.06	2.50	202.50	0	0.00
	Morus alba	native	5	0.31	8.50	752.50	1	1.84
	Parthenocissus quinquefolia	native	1	0.06	0.00	102.50	0	0.00
	Quercus alba	native	2	0.13	7.50	505.00	2	5.73
	Quercus marilandica	native	1	0.06	2.50	202.50	0	0.00
	Quercus rubra	native	4	0.25	5.63	535.00	0	0.00
	Rubus odoratus	native	1	0.06	5.00	302.50	0	0.00
	Toxicodendron radicans	native	1	0.06	2.50	202.50	0	0.00
	Viburnum prunifolium	native	7	0.44	3.57	560.36	0	0.00
Providence Park, DC								

Medium									
N39.51	W-78.49	*Acer negundo*	native	1	0.06	10.00	502.50	0	0.00
		Acer pensylvanicum	native	1	0.06	10.00	502.50	1	2.33
		Acer platanoides	invasive	5	0.63	4.50	592.50	1	1.89
		Acer rubrum	native	7	0.88	4.64	703.21	8	13.78
		Carpinus caroliniana	native	2	0.25	18.75	955.00	0	0.00
		Dioscorea oppositifolia	invasive	1	0.06	7.50	402.50	0	0.00
		Fraxinus pennsylvanica	native	2	0.13	5.00	405.00	1	2.23
		Liriodendron tulipifera	native	8	0.50	5.00	720.00	12	19.58
		Lonicera japonica	invasive	1	0.13	2.50	202.50	0	0.00
		Parthenocissus quinquefolia	native	1	0.06	5.00	302.50	1	1.89
		Prunus serotina	native	1	0.06	2.50	202.50	2	5.26
		Robinia pseudoacacia	native	1	0.06	5.00	302.50	2	4.92
		Rosa multiflora	invasive	6	0.38	4.17	681.67	1	1.70
		Rubus odoratus	native	3	0.38	2.50	407.50	0	0.00
Dawson Terrace, DC									

High									
N39.54	W-78.28	*Acer platanoides*	invasive	6	0.38	3.75	665.00	5	8.25
		Acer negundo	native	1	0.06	7.50	402.50	2	5.05
		Acer rubrum	native	2	0.13	3.75	355.00	8	15.62
		Asimina triloba	native	1	0.06	2.50	202.50	3	6.71
		Cercis canadensis	native	1	0.06	2.50	202.50	3	6.71
		Dioscorea oppositifolia	invasive	2	0.13	17.50	905.00	0	0.00
		Euonymus altus	invasive	4	0.25	5.63	635.00	0	0.00
		Fraxinus pennsylvanica	native	3	0.19	6.67	574.17	7	13.18
		Hedra helix	invasive	2	0.13	1.25	255.00	0	0.00
		Ilex opaca	native	1	0.06	10.00	502.50	0	0.00
		Liriodendron tulipifera	native	3	0.19	4.17	474.17	3	10.99
		Lonicera japonica	invasive	2	0.13	7.50	505.00	0	0.00
		Parthenocissus quinquefolia	native	1	0.06	5.00	302.50	3	5.19
		Paulownia tomentosa	invasive	1	0.06	2.50	202.50	0	0.00
		Prunus serotina	native	2	0.13	2.50	305.00	4	7.49

	Ptelea trifoliata	native	3	0.19	6.67	574.17	3	5.58
	Robinia pseudoacacia	native	1	0.06	7.50	402.50	0	0.00
	Rosa multiflora	invasive	2	0.13	10.00	605.00	2	3.57
	Sassafras albidum	native	2	0.13	6.25	455.00	2	3.86