Ozone

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Ozone is the product of chemical reactions that take place in the lower atmosphere when volatile organic compounds mix and react with nitrogen oxides in the presence of sunlight. Ozone exposure, uptake, and sensitivity all play important roles in the response of plants to ozone. Weather patterns and changes in emissions of precursors account for a majority of year-to-year variations in ozone exposure. The uptake of ozone by plants depends upon many things, including physiological age, climate, light availability, site characteristics, and available soil moisture (Peterson and others 1993, Bartholomay and others 1997, Samuelson and Kelly 2001). Sensitivity to ozone varies by species, genotype, physiological age, and leaf morphology. Several species, such as black cherry (Prunus serotina Ehrh.) and blackberry (Rubus allegheniensis Porter), are known to be sensitive to ozone and exhibit a visible foliar response. In addition, reduced growth and decreased species richness have been reported from studies of ozone impacts to plants (Arbaugh and others 1998, Barbo and others 1998, Bartholomy and others 1997, McLaughlin and Downing 1996, Rebbeck 1996, Reinert and others 1996, Samuelson and Edwards 1993, Somers and others 1998).

Despite the seemingly large amount of research on ozone exposure and ozone impacts to plants, there are still several key unknowns (see reviews by Bytnerowicz 2002, Chappelka and Samuelson 1998, Karnosky and others 2007, Kickert and Krupa 1990, Krupa and others 2000, Samuelson and Kelly 2001). The more elusive issues are the relationship between ambient ozone and foliar injury, the relationship between foliar injury and physiological response (i.e., reduction in photosynthesis), and the effect of ozone on growth. Complicating the exposure-response issue is the existence of multiple ozone exposure indices (i.e., SUM00, SUM06, W126) and the uncertainty surrounding variation in ozone exposure at the regional, local, and microsite scale. The SUM00 index is calculated by multiplying all hourly concentrations by time. Similarly, the SUM06 index is derived by multiplying the number of hours when the ozone concentrations stayed above 0.06 parts per million (ppm). The W126 is a weighted index with higher concentrations having higher weights (Bytnerowicz and others 2008).

Further complicating this issue is the questionable usefulness of ozone exposure indices to accurately reflect the effective ozone flux experienced by vegetation. While physiological responses in the form of reduced growth or biomass have been confirmed for some species, these studies have been mainly from controlled chamber studies, which may not directly translate to plant response in the natural environment. In addition, ozone sensitivity within species is quite variable and complicates interpreting results. The result is that cause and effect relationships have been difficult to confirm.

Between 1998 and 2008, seven ozone Evaluation Monitoring (EM) projects were funded under the national Forest Health Monitoring (FHM) Program of the Forest Service, U.S. Department of Agriculture. Five of them fell into the general category of ozone effects on plants. While many species have been tested for sensitivity to ozone, the list is far from comprehensive. Awareness of which species tend to exhibit consistent foliar injury is a key factor in successful large-scale biomonitoring surveys. One project (NE-EM-00-01) was initiated to determine the sensitivity of several species that were suspected to be sensitive based on field observations. Some studies have suggested that visible ozone injury and growth reductions are correlated in some species. The need to test this correlation in situ resulted in four of the EM projects (SO-EM-01-01; NE-EM-99-02; WC-EM-99-05; SO-EM-00-04). By comparing sensitive and tolerant individuals within the same species, three of the studies examined the genotypic variability that exists in ozone sensitivity (SO-EM-01-01; WC-EM-99-05; SO-EM-00-04). One study attempted to determine the impact of foliar injury on photosynthetic capacity (SO-EM-00-04).

Two of the EM projects addressed exposure issues. While a large network of ozone monitors currently exists across the United States, ozone varies across the landscape. Predicting the exposure at the regional level utilizing existing data was the topic of one of the funded projects (NE-EM-98-04). One way to better predict exposure at the local level is sometimes accomplished using passive ozone samplers, which were employed in another project (WC-EM-05-03).
Project NE-EM-00-01: Determining the Ozone Sensitivity of New Bioindicator Species for the Biomonitoring Program

This study was part of a larger project at Pennsylvania State University to evaluate species for ozone sensitivity in controlled fumigation studies and determine validity of field observations. Demonstrating a linkage with the national ozone biomonitoring program of the Forest Inventory and Analysis (FIA) Program of the Forest Service, this project was initiated because field personnel were finding species with ozone-like injury that were not previously recognized as sensitive to ozone. This led the principal investigators to suspect that many more species than previously known develop ozone-induced foliar injury. This project is directly applicable to ozone biomonitoring data collection, because as the number of species increases, so does the likelihood that the crews will find and inspect the minimum number of species at a given site, and this, in turn, increases the number of potential biomonitoring sites.

Thirteen species native to the Northeastern United States were tested for ozone sensitivity in chambers utilizing four repetitions of four ozone exposure treatments [30, 60, 90, and 120 parts per billion (ppb)] and a control (Orendovici and others 2003). At 30 ppb little or no foliar injury was detected on any of the species tested. Ten species began showing symptoms at 60 ppb. Two of the 13 species became symptomatic at 90 ppb. Only one of the species tested did not become symptomatic until the 120 ppb level. Foliar injury was typically chlorotic spotting, upper leaf stippling (most reliable symptom), discoloration, dead patches, reddening, and early leaf death.

Two species, swamp milkweed (Asclepias incarnate L.) and winged sumac (Rhus copallina L.), showed the greatest potential to be ozone bioindicators. However, the authors caution that chamber studies do not always fully represent natural conditions, and these results may need further investigation. One species, pawpaw (Asimina triloba (L.) Dunal), was nonspecific for ozone injury and needs further investigation before drawing conclusions. Of particular note in this study was the variability among seedlings within species (i.e., several seedlings had severe injury, while others were asymptomatic). This may be the result of genetic variation in sensitivity. This project is particularly noteworthy, because the majority of the species tested are now on the FIA supplemental list of bioindicator species and are used to identify areas where ozone injury occurs.

Project SO-EM-01-01: Impact of Ground-Level Ozone on Trees in the Great Smoky Mountains National Park

This *in situ* research was conducted in the Great Smoky Mountains National Park, a Class I area. This designation, established in 1977 as an amendment to the Clean Air Act, affords greater protection to areas such as national parks that are greater than 5,930 acres (2 400 ha) in size. Building on an earlier study (Somers and others 1998), the principal investigator utilized black cherry and yellow-poplar (Liriodendron tulipifera L.) trees to determine if correlations exist (1) between ambient ozone concentrations and radial growth and (2) between ozone foliar injury and radial growth. To accomplish these goals, tree cores were taken from sensitive and nonsensitive individuals of the two species at three different locations within the park. Sensitivity was determined by the degree of foliar injury of each individual tree.

Growth differed significantly between locations for both species. Reasons for this may include site differences or differences in ozone exposure (Somers and others 1998). No growth differences for sensitive versus nonsensitive black cherry were detected. Growth differences between sensitive and nonsensitive individuals of yellow-poplar were not significant. This contrasts with a previous study (Somers and others 1998), when the growth differences between sensitive and nonsensitive yellow-poplar were significant. In that study, growth of sensitive yellow-poplar averaged 0.11 inches (0.27 cm) per year, while that of nonsensitive individuals averaged 0.13 inches (0.34 cm) per year. In the present study, sensitive individuals continued to have slower growth (0.16 inches (0.41 cm) per year) compared to nonsensitive individuals (0.17 inches (0.44 cm) per year), although the difference was not statistically significant. No location/sensitivity interactions were observed for either species.

Growth responses due to ozone exposure were only narrowly established with this study. However, one finding stands out: individuals of some species with foliar injury may respond with reduced growth when compared to uninjured (nonsensitive) individuals, while some species (e.g., black cherry) may not react in the same manner.
Projects NE-EM-99-02; NE-EM-00-02; NE-F-01-03: Ozone Effects on Plant Productivity and Population Dynamics in the Lake States; Ozone Effects on Common Milkweed and Black Cherry Productivity and Injury Dynamics in the Southern Lake States

Conducted in the southern Lake Michigan region, this in situ study demonstrated linkage to the FIA ozone biomonitoring program by utilizing the FIA ozone biomonitoring survey techniques and plots. The main objective was to assess whether ozone-induced foliar injury is correlated with productivity (branch elongation, plant height, or reproduction output) in black cherry and milkweed (Asclepias syriaca L.). This 3-year study utilized a network of 18 plots, with 9 located in an area of low ozone exposure (SUM06 <12 ppm-hours, average = 12.2, peak = 91 ppb) and 9 in an area of high ozone exposure (SUM06 >22 ppm-hours, average = 18.1, peak = 110 ppb). Ozone exposure was estimated using Environmental Protection Agency (EPA) hourly ozone data (Bennett and others 2006). To account for many confounding factors, several site and environmental variables, such as soil properties and rainfall, were included in the analyses.

Black cherry branch elongation was negatively affected by ozone exposure, while the number of seeds, height, and diameter were not, although this was not unexpected for the last two variables (Bennett and others 2006). For milkweed, the number of seedpods per plant was negatively affected by ozone, while plant height was not. The inclusion of the year-to-year and site-to-site variation in rainfall and temperature proved useful in explaining plant responses to ozone. Other variables, such as soil properties, varied significantly between the two ozone regions (especially percent nitrogen) and also were integral to the analyses. For black cherry, rainfall, percent soil nitrogen, and mean temperature departure were more important than ozone exposure for branch elongation. For milkweed, ozone exposure was more important than rainfall for percentage of stems with pods, but for height total soil nitrogen was more important, followed by ozone exposure.

This study emphasizes that different species have different ozone-responsive productivity measures, and that species respond to different environmental variables. In addition, rainfall and percent nitrogen in the soil proved to be possible interacting factors, highlighting the need to consider a wide variety of environmental variables when studying ozone exposure and productivity responses. Another important aspect of this study was the response to different ozone measures for the two species. Black cherry branch elongation responded to SUM06 ozone exposures greater than 13.3 ppm-hours. In comparison, milkweed height and pod formation responded to peak 1-hour

concentrations greater than 93 ppb and 98 ppb, respectively. The foliar ozone injury, which differed significantly between the low and high ozone sites, is the topic of a study currently in progress.

Project WC-EM-99-05: Evaluation of Ozone Foliar Injury and Bole Growth of Pines in Southern California

This study, located in the southern Sierra Nevada (Sequoia and Sierra National Forests), utilized data from plots that were installed in 1977 and 1978 and then measured every other year through 2000. The primary objective was to determine if trees with severe crown injury had different growth rates than trees with little or no crown injury. Both ponderosa pine (Pinus ponderosa Dougl. Ex Laws.) and Jeffrey pine (P. jeffreyi Grev. and Balf.) were studied. Presumably, these pines have similar ozone sensitivities, but there is some evidence that growth is correlated with ozone exposure for Jeffrey pine but not for ponderosa pine. Genetic differences, site, age, and stand dynamic differences may account for some of this. In California some ponderosa pine stands are located in areas with high nitrogen deposition, while Jeffrey pines tend to be located on sites with lower nitrogen deposition. This may partly explain the differences in growth response, as there is some evidence that the positive effects of nitrogen deposition can mask the negative effects of ozone exposure.2 The analysis was done using data from cores that were obtained in 2000 from 15 pairs of trees (on 14 sites) based on historical and present crown position, present diameter at breast height, and average 20-year crown injury. Both severe and slight injury groups had significantly higher pre-pollution period basal area growth rates. Severely injured trees had significantly lower basal area growth after 1955 (relative to slightly injured trees), but changes in growth between time periods was not significantly different for the two groups of trees.

Project SO-EM-00-04: Ozone Impact on Sensitive Forest Tree Species

The objectives of this in situ project which took place in Giles County, VA, were to compare the photosynthetic capacity of tolerant and sensitive genotypes of black cherry exposed to ambient ozone concentrations and relate visible injury to carbon assimilation capacity. To achieve these goals, a variety of measurements were taken, including maximum net photosynthetic rate (Pnmax), stomatal conductance (gas exchange rates), chlorophyll fluorescence, chlorophyll content, and visible foliar injury.

Chlorophyll fluorescence revealed that ozone affected photosystem II (PSII) activity by reducing the maximum photochemical efficiency of PSII in sensitive black cherry by between 5 and 8 percent. In addition, the electron transport rate through PSII was significantly lower (28 to 55 percent) in sensitive trees compared to tolerant ones. Although chlorophyll content was the same between genotypes early in the season (June), by July, sensitive black cherry trees had 48 percent less chlorophyll than tolerant ones. In addition, as leaf injury increased, chlorophyll concentrations decreased ($R^2=0.95$) compared to noninjured leaves. At 25 percent leaf area injured, there was a 70 percent reduction in chlorophyll content.

Differences between gas exchange rates of sensitive versus tolerant trees were inconsistent across the study period. During 2001, stomatal conductance rates were the same for the two genotypes. In contrast, during 2003, conductance rates were 30 percent higher in tolerant black cherry in July but were 10 percent lower in August. This may, in part, have been due to the variation in weather and ozone exposure that occurred across the study period. In 2001, the early part of the summer was cool and relatively wet, while the later part was warmer than average and moderately dry; ozone concentrations were moderate. The year 2003, however, was extremely wet and cool with only moderate to low ozone exposure.

There was a significant progressive decline in $P_{n\text{max}}$ of between 12 and 65 percent, in sensitive trees, compared to tolerant trees. In addition, $P_{n\text{max}}$ was correlated with leaf age and amount of visible injury. When young leaves were compared to mature leaves, $P_{n\text{max}}$ was reduced by 25 percent in tolerant trees and by 50 percent in sensitive trees. At 5.5 percent of leaf area with injury, $P_{n\text{max}}$ was reduced by 10 percent, and at 45 percent injury, it was reduced by 80 percent when compared to noninjured leaves.

In addition to reduction of $P_{n\text{max}}$, both the quantum efficiency for CO$_2$ fixation and the carboxylation efficiency were significantly reduced between June and August for sensitive trees compared to tolerant ones, and thus both the light and dark reactions of photosynthesis were affected by ozone. Even in 2003, a year of fairly low ozone concentrations, carboxylation efficiency was 24 to 26 percent less in sensitive versus tolerant trees, highlighting the fact that under even low ozone concentrations carboxylation processes may be affected substantially by oxidative stress. Early in the summer, maximum carboxylation was 25 percent higher in tolerant trees than in sensitive trees; however, by August, it was 250 percent higher, leading the author to conclude that the impact of ozone was much more severe on the dark reactions. In experiments conducted in 2003, under saturating CO$_2$, the author determined that by late summer (August) there was 31 percent less ribulose biphosphate regeneration capacity in sensitive versus tolerant trees. This means that sensitive trees had an approximately 25 percent less total capacity to fix CO$_2$ into sugars than tolerant trees.

The author concluded that ozone affected both the light and dark reactions of photosynthesis in sensitive black cherry. Although the impact to photosystem activity was substantial, the effect was greatest on carboxylation. The impact of ozone on photosynthetic activity could ultimately affect biomass accumulation, growth, and reproductive capacity.

**Project WC-EM-05-03: Intensified Ozone Monitoring and Assessment of Ozone Impacts on Conifers in Southern California**

To better understand ozone distribution and phytotoxic potential in the San Bernardino Mountains (SBM), this multi-year project used a variety of funding sources to install a network of passive samplers in the study area. These samplers allow ozone distribution to be characterized at the landscape or forest stand level. The linkage to the FIA ozone biomonitoring program presented the opportunity for the researchers to fill data gaps in areas where ozone biosites are scarce.

This study revealed that ozone concentrations in the SBM, although currently lower than in the 1960s and 1970s, are still some of the highest in the United States (Byternowicz and others 2008). Ozone concentrations increased from east to west. Ozone levels and distribution were fairly stable during the study period (2001-06). The ozone phytotoxic potential in the SBM, particularly in the Central and Eastern Transverse and Peninsular Mountain Ranges, is the highest among mountainous regions in the United States and Europe. The study also demonstrated that high elevation sites have a different diurnal pattern than low elevation sites. Ozone concentrations do not fall at night at the high elevation sites as they do at the low sites. This study also highlights the phytotoxic potential that currently exists in the SBM, despite decreasing levels of ozone.

**Project NE-EM-98-04: Estimating Seasonal Exposures & Concentrations of Ozone for the Great Lakes Region**

This study was initiated to aid in the determination of ozone exposures in the Great Lakes Region. Unfortunately, no
detailed information was available regarding this project. There was, however, reference to this project in the NE-EM-99-02 proposal: “ozone exposure studies conducted for the FHM program … would be used to guide the site selection process.”

Summary of Key Findings

**NE-EM-00-01**—Many more species than previously known develop ozone-induced foliar injury under field conditions. Of particular note in this study was the variability among seedlings within species (i.e., several seedlings had severe injury, while others were asymptomatic). There was a strong association between cumulative ozone exposure and leaf injury; however, the amount of exposure required to cause symptoms and the progression of injury differed significantly between species.

**SO-EM-01-01**—No growth differences for sensitive versus non-sensitive black cherry were detected. Growth differences between sensitive and nonsensitive individuals of yellow poplar were not significant. Individuals of some species with foliar injury may respond with reduced growth when compared to uninjured (nonsensitive) individuals, while some species may not react in the same manner.

**NE-EM-99-02; NE-EM-00-02; NE-F-01-03**—This study emphasized that different species have different ozone-responsive productivity measures, and that species respond to different environmental variables. In addition, this study highlighted the need to consider a wide variety of environmental variables when studying ozone exposure and productivity responses. Another important aspect was the response to different ozone measures for the two species measured.

**WC-EM-99-05**—For the species tested, both the severe and slight injury groups had significantly higher pre-pollution period basal area growth rates. Severely injured trees had significantly lower basal area growth after 1955 (relative to slightly injured trees), but changes in growth between time periods was not significantly different for the two groups of trees.

**SO-EM-00-04**—Ozone affected both the light and dark reactions of photosynthesis in sensitive black cherry. Even under low ozone concentrations, carbon reduction processes may be affected substantially by oxidative stress. Although the impact to photosystem activity was substantial, the effect was greatest on carboxylation. The impact of ozone on photosynthetic activity could ultimately affect biomass accumulation, growth and reproductive capacity.

**WC-EM-05-03**—This study revealed that ozone concentrations in the San Bernardino Mountains, although currently lower than in the 1960s and 1970s, are still some of the highest in the United States. The study also demonstrated that high elevation sites have a different diurnal pattern than low elevation sites.

Utilization of Project Results

Project NE-EM-00-01 is particularly noteworthy, because 10 of the species tested are now on the FIA supplemental list of bioindicator species and are being used to identify areas where ozone injury is occurring. The confirmation of ozone sensitivity of these species under controlled exposure conditions proves cause and effect and validates the usefulness of these plants for biomonitoring practices. More species means more data records by plot and FIA region, thus improving the reliability of the plot-level injury index. The project findings were also used by researchers associated with the National Park Service to generate ozone sensitive plant lists for the assessment of foliar ozone injury on national parks (Porter 2003). From a policy perspective, FIA biomonitoring results, including data collected on the new species, helped to inform EPA staff in the most recent scientific review of the national ambient air quality standards for ozone. Furthermore, the Heinz Center for Science, Economics, and the Environment has used the biomonitoring data to develop quantitative tools for the benefit of natural resource managers responsible for the assessment of ecosystem response to changes in ozone air quality.

Suggestions for Further Investigation

Despite the wealth of knowledge that exists, and the obvious contribution that the funded EM projects have made, the effect of ozone on forest health is still not fully understood. As noted by investigators in project WC-EM-05-03, better biologically based indices of the phytotoxic potential of ozone are needed. The current network of ozone monitors does not adequately address the spatial and temporal variation in ozone exposure, particularly at the microsite scale (i.e., ozone exposure to trees in closed canopy forests). Another primary research need is a better understanding of the effective ozone flux to plants. Whether the primary issue is stomatal conductance, or other factors, as suggested in project SO-EM-00-04 and by Bagard and others (2008), needs investigation. Also needed are studies that measure the metabolic potential of plants to detoxify ozone and repair damage.

As suggested by EM project SO-EM-00-04, determining the effect of ozone on different aged leaves exposed to ozone for different amounts of time would improve estimates of ozone...
effects on canopy level photosynthesis and allow biomass loss estimates to be linked with visible injury. In addition, the increased impact of ozone to older leaves and the timing of episodes of high ozone exposure have implications for determining critical ozone levels.

EM projects NE-EM-99-02, NE-EM-00-02, and NE-F-01-03 underscore the need to include environmental variables in ozone studies. Their results suggest that studies investigating the role of rainfall and percent soil nitrogen in ozone injury are needed. In addition, they noted that the investigation of additional soil properties (i.e., magnesium, manganese, and copper) would be beneficial. The correlation between individual plant productivity and foliar ozone injury within a given ozone exposure also needs investigation. That black cherry responded to seasonal SUM06 values and milkweed to short-term peak values emphasizes the need to better understand the relative importance of long-term versus short-term ozone exposures, an issue that has been much debated.

Project SO-EM-01-01, which resulted in somewhat conflicting results from the previous study it was built on, emphasizes that long-term studies of ozone injury, exposure, and tree growth are needed. In addition, studies of growth rates in relation to ozone and climatic records should be conducted.

That chamber studies do not always fully represent natural conditions emphasizes the need for continued investigation of species sensitivities (NE-EM-00-01). In addition, there are many species that need further evaluation, such as pawpaw (as noted in NE-EM-00-01), while others, such as, southern red oak (Quercus falcata Michx.) and sweet birch (Betula lenta L.) have yet to be tested for ozone sensitivity.

The effect of the interaction between ozone and nitrogen is another area that needs investigation. Some research has shown that nitrogen may reduce sensitivity to ozone, while other studies have shown negative impacts, depending on the timing of exposure (i.e., sequential versus simultaneous) (Bytnerowicz 2002). In addition, whether nitrogen is supplied via the soil or the air as nitric acid vapor or ammonia could result in a different response.


Literature Cited
