Ozone Pollution Impacts on Native Trees and Wildflowers in Great Smoky Mountains National Park

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Final Report
June 1, 2006

Title: Ozone Pollution Damage to Growth and Physiology of Native Trees and Wildflowers in Great Smoky Mountains National Park

FINAL REPORT

Project Number: NPS #: D-609 PMIS #: 66941 **Region: SERO Park: GRSM**
Service-wide Goal Number: Ia3 (Air Quality)
Date Plan Prepared: March 31, 2002 for FY01-FY05
Date Final Plan Submitted: June 1, 2006

Organizations Involved in Project:

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- **Appalachian State University, Boone, North Carolina**
- **University of Tennessee, Knoxville, Tennessee**
- **Great Smoky Mountains National Park, Tennessee/North Carolina**

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Project Summary

The purpose of this project was to obtain a significant new understanding of the impacts of ozone pollution on selected tree and wildflower species in Great Smoky Mountains National Park (GRSM). The objectives of the study were addressed through 1) tree ring analyses to relate tree growth to past ozone concentration and climate variations, 2) high resolution analyses of daily growth patterns of mature trees related to variations in ozone concentration and climate, 3) determination of the relationship between ambient ozone concentrations and growth and reproduction of native wildflowers growing in GRSM (both *in situ* and in bioindicator gardens (bioindicators are ozone sensitive species used to detect the presence of phytotoxic ozone levels in the field)), and 4) education of the public to the rationales, approaches, and results of the studies through the *Parks as Classrooms* program implemented with strategic placement of bioindicator gardens with ozone-sensitive species in GRSM.

- Regarding Objective 1, trees were assigned to an ozone-sensitivity group (symptomatic or asymptomatic) at the start of the project based on previous foliar injury surveys. Twenty trees/species /site (10 symptomatic and 10 asymptomatic trees, 60 trees per species, 120 total trees, 240 samples) were cored in October 2001. No discernible relationship could be demonstrated regarding foliar injury and radial tree growth of yellow-poplar (*Liriodendron tulipifera*) and black cherry (*Prunus serotina*) for the periods 1997-2001, 1992-2001 and 1987-2001. However, radial growth of the symptomatic yellow-poplar was less than that of the asymptomatic trees. In an earlier coring study at GRSM, Somers *et al.* (1998) found that radial growth of symptomatic yellow-poplar was significantly less than that of asymptomatic trees over a 5 and 10-year period (1990-1994 and 1985-1994). The radial growth difference between symptomatic and asymptomatic yellow-poplar was less significant in this study than in the Somers et al. (1998) study. Several factors could account for the difference in significance including a loss of ozone-sensitivity over time, differences in collection methods, and differences in sample size (fewer trees sampled in 2001). In addition, we could not find any significant changes in growth over time directly related to ozone levels. A possible reason for this failure is the need to evaluate a longer growth period. Because ambient ozone concentrations were not monitored at GRSM prior to 1980, and most ozone monitoring sites in the park began operation after 1987, it is not possible to compare pre-1987 tree rings to ozone concentrations. Future modeling efforts of ozone concentration vs. radial growth are warranted.

- Based on manual and automated dendrometer band data (Objective 2), our results indicate that ozone has the potential to cause reductions in both seasonal and daily growth of selected trees. In addition, models that have been developed using these data (McLaughlin, personal communication) indicate that a significant growth reduction of mature forest trees (ca 50%) during high ozone years results from a combination of ozone with increased water demand potentially resulting from stomatal dysfunction. Associated losses in soil water are also having significant effects on late season streamflow based on these models. These results are contrary to other reports in the literature, primarily derived from short-term elevated ozone exposures of plants growing in pots or chambers that indicate ozone exposure causes stomatal closure. The multiple effects of ozone and

water availability on the growth of mature forest trees are expected to have many and varied negative implications for forest ecosystem health and function. Current regional predictive models regarding ozone effects on forest growth need to take these data into account. Water use was not correlated with ozone sensitivity during a one-year study (2004) of yellow-poplar trees. This is most likely due to the near record low ozone concentrations during that year, or to other unknown factors.

- Regarding Objective 3, numerous investigations were conducted during the course of the project, and our results indicated that ozone can have a profound effect on native wildflowers growing in GRSM. Previous studies in the Park have concentrated on visible injury assessments, whereas our current efforts were focused on gaining a better understanding of the physiological, biochemical and environmental factors that govern ozone sensitivity. The majority of our research was with cutleaf coneflower (*Rudbeckia laciniata*) (an ozone-sensitive species). In general, ozone had a profound effect on these factors, although given the limited scope of the project, further investigations are needed. Some major conclusions were:

    - 1) Gas exchange differed by ozone sensitivity class (asymptomatic vs symptomatic plants based on foliar injury observations during the growing season), with photosynthesis being reduced in the severely-injured ozone-symptomatic clones. There was a wide range in stomatal response to changes in both vapor pressure deficit (VPD) and light, but ozone-sensitive plants were more variable in their response, and the response also decreased in magnitude at higher VPDs. Stomatal conductance of asymptomatic plants was greater, not lower, than ozone-symptomatic plants over a broad range of steady-state light levels. However, when there was no foliar injury evident, both classes of plants had similar rates of photosynthesis and stomatal conductance.

    - 2) Seed heads were collected from symptomatic and asymptomatic cutleaf coneflower at the end of the 2004 growing season to determine if reproductive effort was affected by development of ozone-induced foliar injury symptoms. There were no differences between sensitivity groups regarding plant height or number of leaves, but symptomatic cutleaf coneflower produced fewer flower heads per plant, and fewer seeds per flower head than the asymptomatic plants. There were no differences in the mean weights of individual seeds. On a per plant basis, however, sensitive plants produced 50% fewer seeds than insensitive plants.

    - 3) To gain an understanding of the physiological effects of ozone within a plant community, we characterized development of cutleaf coneflower canopies over the growing season. Measured values near the ground were approximately 50% of the ozone levels measured 1 m above the stand. A model was then developed to predict ozone deposition within a canopy of cutleaf coneflower. Predictions of patterns of ozone distribution within the canopy by the model were compared with actual observations throughout the canopy. In general, the model accurately predicted changes in ozone concentrations within the canopy, but over-predicted ozone levels at lower concentrations near ground-level, possibly due to scavenging by unknown sources near the ground. Further refinement of the model is needed.

In addition to the objectives addressed with NPS funding, several ancillary (pilot) studies were conducted that provide useful information for park managers.

- One study attempted to determine if any fluorescence parameters are good predictors of plant responses to ozone. Preliminary analyses suggest that the area above the fluorescence curve, which is a measure of the size of the plastoquinone pool (a part of the electron transport system) is substantially reduced in symptomatic leaves, and is highly correlated with the maximum rate of photosynthesis. This technique may offer a simple way of determining the magnitude of photosynthetic impairment due to ozone without resorting to using a more complicated gas exchange system.

- Measurements were made of the extracellular antioxidant activity in leaves from symptomatic and asymptomatic cutleaf coneflower, crown-beard (*Verbesina occidentalis*) and tall milkweed (*Asclepias exaltata*) in an attempt to relate ozone sensitivity with the inherent antioxidant capacity of several native wildflowers growing in GRSM. Greater quantities of ascorbic acid in leaf tissue were found in tall milkweed than in either crown-beard or cutleaf coneflower. Overall, distinct differences in antioxidant metabolism were found in the wildflower species that could differentially affect response to ozone stress. These data will help determine if differentiation in ozone sensitivity is related to potential differences in antioxidant activity in native wildflowers. Chlorophyll levels are often reduced after ozone exposure. Symptomatic leaves of cutleaf coneflower were found to contain lower chlorophyll levels than asymptomatic leaves. A current method used to facilitate the nondestructive measurement of chlorophyll in leaves is the Minolta 502 greenness meter (SPAD meter). Although the meter can be accurately calibrated against actual chlorophyll amounts, we have discovered that this calibration changes in leaves with moderate to severe amounts of ozone injury. Factors associated with the development of leaf symptoms (different pigments, collapse of dead cells, etc.) may interfere with the operation of the meter. This is an important finding since the SPAD meter is used quite frequently to obtain relative measures of leaf chlorophyll content.

- Preliminary genetic analyses [Random Amplified Polymorphic DNA (RAPDs)] from a previous study conducted in GRSM indicated symptomatic and asymptomatic individuals of cutleaf coneflower at Purchase Knob to be genetically distinct, whereas at Clingmans Dome, environment appears to play a more important role, *i.e.*, only two separate genotypes were found. These differences are probably related to the age of the population, and whether or not the plants were produced vegetatively by rhizomes or sexually by seed. To better understand the genetics of ozone sensitivity of cutleaf coneflower, leaf samples from symptomatic and asymptomatic plants in 23 different populations in the Park, primarily in the vicinity of Clingmans Dome and Purchase Knob, were collected. These data are currently being analyzed to determine whether or not the observed sensitivity is directly related to genetic differences or environment.

- An experiment to document the effects of drought on the development of ozone injury in the cutleaf coneflowers at Purchase Knob was initiated in the spring of 2004. The unusually wet and cool weather made that endeavor difficult. However, transpiration rates of previously-identified symptomatic and asymptomatic plants were monitored, and the data indicate of trend of reduced whole-plant water loss rates in symptomatic plants with visible foliar injury compared to asymptomatic plants. This means that their was less stomatal opening on the symptomatic plants. However, because of the climatic conditions no inferences could be made regarding an alteration in drought susceptibility.

- To determine the nutritional quality of injured versus uninjured cutleaf coneflower leaves, leaves were collected at three locations in the Park during July 2004. Preliminary analyses indicate that injured leaves contain less nitrogen and less food value to animal herbivores. In addition, it appears that a strong relationship is evident between the production of total phenolics and lignin for the injured leaves, but not as strong for uninjured ones. Increases in these secondary compounds have implications related to plant defense, ie., the greater the amount of these compounds generally results in a lower relative food value for ruminant herbivores. The implication of theses results on animal nutrition is currently being explored in more detail.

Regarding Objective 4, bioindicator gardens were established as part of the project, and turned over to GRSM personnel during 2003. The garden at Purchase Knob has been utilized for several educational events, including training sessions, and teacher and student workshops. This concept is currently being expanded to other NPS units and to schools in the area and in other parts of the country.

Introduction

Problem Statement

Air pollution is a very significant threat to the natural resources and public health in Great Smoky Mountains National Park (GRSM). Great Smoky Mountains National Park has the highest cumulative ozone pollution exposures of any National Park in the eastern United States and ozone levels increased dramatically during the decade of the 1990s (U.S. Environmental Protection Agency's [U.S. EPA Aerometric Information Retrieval System (AIRS) database and Jim Renfro, personal communication]. This widespread air pollutant has been reported to cause injury to native vegetation in the Park (Neufeld *et al*. 1992, Chappelka *et al*. 1997, Chappelka *et al*. 1999), and is potentially affecting public health, including that of both park visitors and staff (Jim Renfro, personal communication).

Tropospheric ozone is a globally-increasing pollutant of worldwide concern because of its implications for both human and ecological health (US EPA 1996). Many species of plants, including forest trees and native wildflowers, experience visual injury to foliage at current ambient levels of ozone (US EPA 1996, Chappelka and Samuelson 1998). A long standing issue among ecologists has been the significance of these visual changes at the leaf-level to subtle shifts in plant growth, competition, and interactions with the biotic and abiotic environment that are expressed at the whole plant level (Heck and Cowling 1997, Sandermann *et al*. 1997). These uncertainties pose an even greater risk for future ecological assessments in light of recent forecasts of increasing stress from a potentially hotter, drier climate (Joyce *et al*.1990), increasing regional ozone exposures (Chameides *et al*. 1994) and urbanization (Brown et al. 2005) in the eastern US in the next several decades.

Results from our project provide critically needed data on physiological and growth responses of mature trees and wildflowers in the field under a range of ambient ozone pollution exposure regimes. This information has been identified as critical for the assessment of ozone pollution effects on natural ecosystems, including those, like GRSM, that are designated as Class I air quality areas under the Clean Air Act. This information can be used by Park management when fulfilling Clean Air and Organic Act responsibilities. Our results, although specific to GRSM, should be applicable to other national parks in the eastern US.

Background

Great Smoky Mountains National Park is the most visited national park in the US, receiving more than 10 million visitors annually, and is also recognized as an International Biosphere Reserve and World Heritage Site. The Park encompasses an area of over 206,000 hectares in the states of Tennessee and North Carolina, and contains a wide diversity of plants and animals representative of a large region of the eastern US. There are more wildflower species in GRSM than in any other national park, and more tree species than in all of northern Europe (Uhler, 2002). Over half of the old-growth forests in the eastern US are found in the Park, and over three-fourths of the spruce-fir ecosystem in the southern Appalachians is located in GRSM (http://www.great.smoky.mountains.national-park.com/info.htm). Therefore, any detrimental effects to vegetation in the Park are of major concern for the entire region (Shaver *et al*. 1994). Great Smoky Mountains National Park experiences high levels of ozone pollution, producing well documented symptoms of visible injury on certain species of vegetation (Neufeld *et al*. 1992, Chappelka *et al*. 1997). In addition, recent work has linked growth losses of mature trees in the field to episodes of high ozone (McLaughlin and Downing 1995), further emphasizing the point that to more fully understand ozone impacts on plant communities of the southeastern US, we will need a greater understanding of species differences in ozone sensitivity, and ozone-climate interactions.

Because of the great topographical relief existing in GRSM, widely different ozone exposure patterns can be found at different locations and elevations (Mueller 1994). These patterns of exposure are critical to determining plant response (Chappelka and Samuelson 1998), but relatively little research has been conducted on the influence of these differences (US EPA 1996). An understanding of these factors is also crucial for extrapolating ozone influences to a wide-spread geographic basis.

Specific Objectives to be Addressed

Our major objectives were to:

1. Determine the relationship between foliar ozone injury and growth for selected tree species in GRSM,
2. Establish the role of seasonal ozone exposures in influencing growth and whole tree water use of mature forest trees under field conditions,
3. Determine the relationship between ambient ozone concentrations and the growth and reproduction of native wildflowers growing in GRSM (both *in situ* and in bioindicator gardens), and
4. Create ozone-bioindicator gardens at selected locations in the Park. These gardens will be a part of the *Parks as Classrooms* program, which will be developed for the general public, middle and junior high school students, and science teachers.

The theme that links these objectives is that a practical understanding of ozone impacts in GRSM requires a broad perspective, ranging across plant life-forms (wildflowers to trees), successional stages (early to late), and life histories (deciduous to evergreen). To accomplish this, we proposed to first define the magnitude of the problem, explore the general physiological mechanisms behind these responses, and finally to perform educational outreach for the public benefit (bioindicator gardens). These goals were primarily met through development of the specific studies described below. Results from our project provide critically needed data on physiological and growth responses of mature trees and wildflowers in the field under a range of ambient ozone pollution exposure regimes. This is information that has been repeatedly identified as essential for the assessment of ozone pollution effects on the growth and productivity of natural ecosystems, including those in Class I air quality areas such as GRSM.

Objective 1: Foliar Injury and Tree Growth

The purpose of this portion of the study was to evaluate the longer-term responses of yellow-poplar (*Liriodendron tulipifera*) and black cherry (*Prunus serotina*) trees previously evaluated in 1995 for visible injury and patterns of radial growth as reported by Chappelka *et al*. (1999) and Somers *et al*. (1998). Since ozone exposures increased dramatically in GRSM from

1990-2000, we expected to find either similar or enhanced effects on tree ring widths, assuming that the reductions found previously were really due to ozone uptake.

Methodology

To observe relationships between ambient ozone concentrations and tree responses, field plots were established in 1991 in the vicinity of three of the Park's air quality monitoring stations: Cove Mountain (elev. 1265 m), Look Rock (elev. 823 m) and Twin Creeks (elev. 597 m). All monitors were located on the northern periphery of the Park (Tennessee). The presence of an ozone monitor at each site permitted correlations of ozone exposure statistics with observed biological effects. Selection criteria are detailed in Chappelka *et al.* (1999). To determine if ambient ozone concentrations were affecting the growth of two important tree species in the southern Appalachian region of the US, yellow-poplar and black cherry trees located within 2 km of ambient ozone monitors, and previously identified regarding ozone sensitivity (Chappelka *et al.* 1999, Somers *et al*. 1998), were re-cored in 2001. Our analysis assumes that the ozone effect on growth can be evaluated by comparing symptomatic and asymptomatic trees growing in close proximity to each other, since no sites in the field are ozone-free. This portion of the study was in concert with an existing pilot project funded by the USDA Forest Service, the purpose of which was to determine if the growth trends observed in the initial study (1995) were still evident. Additional data were collected to verify visible foliar symptoms with growth. The following types of data, collection devices, and approximate measurement resolution were used in this study:

Data collection – tree cores - Twenty trees/species /site ((10 symptomatic and 10 asymptomatic trees per location, 60 trees per species, 120 total trees, 240 samples) were cored in October 2001. Two cores were taken from each tree at breast height (1.4 m) at right angles. The samples were sent to Appalachian State University for measurement of ring widths. Cores were dried, mounted, and then sanded. Data were cross-dated and measured using dissection scopes and commercial tree-ring software. All cores with unusual data were checked for potential errors. As part of standard laboratory QA methodology, 10% of the cores were randomly selected and remeasured. Growth ring data from the 1995 study were compared with the 2001 data to determine the accuracy between the two collection periods.

Data collection – potential competitors – Potential competitors were measured during April 2002 using a 1.15 m^2/ha angle gauge to select trees in proportion to their diameter and inversely proportional to the distance from each tree (see Somers *et al*. 1998 for more details). In addition, all sample trees were measured for tree height, diameter, crown condition and the presence of insect pests or diseases.

Data collection – ozone and climate data – Using the US EPA AIRS network and NPS Air Quality database temperature, rainfall, ozone and solar radiation data from several stations within the Park (varies by year) were collection. We attempted to collect data from as many time periods as possible, but only could gather quality data from 1980 – present for most variables examined.

Statistical analysis – Radial growth for each species based on five-year increments, were initially analyzed using a two-way analysis of variance (site and sensitivity are assumed to be fixed variables). Covariate analysis (sensitivity class and competitors) were used as appropriate (see Somers *et al.* 1998 for more details). Predictive equations were developed using multivariate techniques and principal components analysis (Somers *et al.* 1998). These data were then compared with growth trends during the previous evaluation period (1990-1994) to determine if differences existed. Various correlation, regression and time-series analytical techniques were used to relate ozone concentrations and indices (SUM06, AOT40, day vs. night, etc.) with growth of the selected tree species.

Results

Results for 5-year radial growth in 1994 (Somers *et al*. 1998) indicated that symptomatic yellow-poplar (visible injury, Chappelka *et al*., 1999) exhibited significantly less radial growth over a 5 (1990-1994) and 10-yr (1985-1994) period when compared with the asymptomatic trees. In order to compare our 2001 results with the previous ones (Somers *et al*. 1998) the first test performed was a simple ANOVA investigating differences between locations and sensitivity to ozone. On the basis of these results ($p= 0.0014$) for yellow-poplar we then proceeded to try to determine if any difference in the two tolerance (sensitive and non-sensitive) classes was due to competition, dbh, height, or age. Since no difference was found in black cherry 5-year radial growth, no further analysis was conducted on this species.

Since some of the trees that were initially sampled in 1994 were not sampled in 2001 (mortality, damage, could not be relocated) we compared the 1994 core data (previously collected with the cores collected in 2001 for similar trees (excluding the missing, dead ones). Based on the 1994 cores, significant differences ($p=0.02$) with regards to sensitivity were observed for yellow-poplar. These results, as expected, were similar to those published in Somers *et al*. 1998. However, using the data from the cores collected in 2001, slightly different results were observed. Significance was observed only at the $p = 0.12$ level. This still indicates a trend between the two sensitivity groups, but it is not as significant as observed in the previous analysis. The reasons for the differences in significance between the two time periods are unknown, but could be due to the following factors: 1) variability due to small sample size, 2) differences in analytical procedures or equipment used between laboratories, and/or 3) differences between individuals reading samples, or (4) there was an actual reduction in the ring-width differences between the two classes of trees, despite the fact that ozone was much higher over this time interval. The original 1994 sample analyses were conducted at the TVA laboratory in Norris, TN. Unfortunately, samples from this collection (1994) were discarded and cannot be directly compared using a single operator.

After comparing samples between collection periods, the next step was to investigate growth patterns for the past 5 years (1997-2001), 10 years (1992-2001), and 15 years (1987-2001). For this analysis all of the 2001 cores were used. As before, there were significant differences between locations, but none between ozone sensitivity groups and no significant interactions.

In the 2001 samples, there was a loss in significance in 5-year radial growth between ozone sensitivity groups compared to the 1994 samples. However, the differences became closer to significance for the 10 and 15-year growth periods for yellow-poplar. The reason was that although ozone-sensitive (symptomatic) yellow-poplar trees maintained a lower average growth rate than asymptomatic trees (0.41 *vs* 0.44 cm, respectively for 5-year growth, 1997-2001), it was no longer statistically significant. It is important to note that the 5-year radial growth from 1990-1994 was 0.27 *vs* 0.34 cm for symptomatic and asymptomatic trees, respectively, which is almost half the growth rate recorded from tree rings measured from 1997-2001. This means that all yellow-poplar trees sampled were growing at a faster rate from 1997-2001 than 1990-1994, and the difference in growth rate between symptomatic and asymptomatic trees had decreased to the point where it was no longer significant. These differences could be related to climatic and site factors occurring during the two time-periods.

We then addressed whether there were significant differences in growth rates from 1990-1994 vs 1997-2001. There were no differences in growth-rates, except by location for yellow-poplar ($p = 0.0007$). As a result, data were pooled across sensitivity groups and locations by species to determine if differences existed among species for the two time periods. Black cherry trees significantly decreased (*t*-test) in growth when the two time periods were compared: 0.52 *vs* 0.44 cm for 1990-1994 compared with 1997-2001, respectively. However, yellow-poplar on average increased in growth during 1997-2001 (0.41 cm) compared with 1990-1994 (0.30 cm). The reasons for these species differences are unknown at present. In summary, 1) black cherry radial growth did not vary by ozone sensitivity groups during any time period analyzed; 2) some differences existed between the original analysis conducted using cores collected in 1994 compared with those collected in 2001; 3) growth for both species was different by location, but no location X sensitivity interactions were observed; 4) yellow-poplar varied by sensitivity group during the period 1990-1994, but not for 1997-2001; and 5) yellow-poplar grew better in 1997-2001 compared with 1990-1994, but the opposite results were found with black cherry.

Since no consistent differences in radial growth patterns were observed, no other analyses (competition, ozone over time, etc.) were conducted. Our initial analyses regarding the relationship between ozone (SUM06, AOT40, etc.), radial growth and climatic trends over time proved inconclusive. We could not find any significant changes in growth over time related to ozone pollution. A possible reason for this failure may be due to a lack of a long-term database regarding trends in ozone, etc. in the GRSM (data only available for approximately 15-20 years at most sites). Using funds from other sources, we are currently attempting to develop various sophisticated modeling techniques in collaboration with Dr. Hanqin Tian (Auburn University) to try to determine if any changes in forest growth in GRSM have occurred over time regarding climate change and ozone pollution. At present, these results are not available and this effort is beyond the scope of the original project objectives.

Objective 2. Effects of Ozone on Mature Tree Growth and Water Use

Methodology

To provide new insights into the physiological and growth impacts of ozone on mature forest trees, we initiated field studies to determine seasonal growth patterns of 90 forest trees at three sites in east Tennessee. In these studies, we used a combination of manual and automated dendrometers distributed across three diverse forested plots to characterize variations in tree growth and tree water use in response to naturally high seasonal and annual variations in ozone and meteorological variables. Our hypothesis was that ambient ozone levels would increase water stress by reducing trees' capacity to recover from the diurnal patterns of water loss and recovery. The forested sites (Oak Ridge, Twin Creeks, and Look Rock) were located near or in

GRSM and were at elevations ranging from 250 m to 750 m. They represented diverse stand histories (severely disturbed in 1999 to undisturbed for > 65 years), and productivity levels (mesic cove hardwood to a more xeric higher elevation ridge site). Previous analyses suggested that episodic ozone exposures of one to three days could interact with soil moisture stress to cause short-term reductions in stem expansion of loblolly (*Pinus taeda*) pine (McLaughlin and Downing, 1995; 1996). However, from these studies mechanisms of response could not be identified due to the lower frequency of the manual measurements employed and the absence of direct measures of water use by the subject trees.

Tree Growth - Our analytical approach in the current studies was to use the manual dendrometer data to describe the similarities and differences among species and years in the seasonal patterns of growth for a larger sample of trees within the area. Manual measures of circumference changes (see McLaughlin and Downing, 1996) at approximate 2-week intervals were recorded for a total of 90 trees across all sites. These trees represented 7 species at Twin Creeks, 8 species at Look Rock, and one species at the Oak Ridge sites. Only yellow-poplar was common to all sites, while red oak (*Quercus rubra*), and pines [pitch (*P. rigida*) and shortleaf (*P. echinata*)] were common to the Twin Creeks and Look Rock sites. Manual band measurements were linked to electromechanical measurements (Agricultural Electronics, Tucson, AZ) of radius changes at 30 minute intervals with automated bands (McLaughlin *et al*. 2003) for 14 trees at the high elevation Look Rock site and 20 trees at the Twin Creeks site. High resolution data for the smaller subset of trees at Look Rock was then used to test for significance of influences of environmental variables on observed seasonal growth patterns and to develop growth models as tools to better understand component influences. Due to interrelationships among environmental variables that follow solar driven diurnal cycles, an important component of our analyses was the development of statistical models to separate and quantify the effects of these variables on stem increment and forest water use.

Tree and Site Water Use - Estimates of whole tree sap flow were made for six trees at both the Look Rock and Twin Creeks sites using Dynamax probes and the heat pulse method (Wullschleger *et al.* 1998). The technique involves determination of temperature differentials between two probes inserted into the sapwood and mounted radially at breast height and approximately 4 cm apart. The rate of transpiration directly influences the rate of heat transfer within the sap stream and provides a measure of mean sapflow velocity. Hourly mean sapflow velocity measurements are normalized to measured sapwood cross sectional area at the probe to obtain sapflow in Kg water dm^{-2} h^{-1} for each sample tree. Because of the importance of vapor pressure deficit (VPD) as a driving force for transpiration and associated sap flow, we also calculated a canopy conductance (sap flow/VPD) to normalize the sapflow data over time.

In addition to these measurements, we compared and contrasted whole-tree sap flow for three symptomatic and three asymptomatic yellow-poplar trees at the Twin Creeks site. Estimates of whole-tree transpiration were made using thermal sap flow probes that consisted of two cylindrical probes of 2 mm diameter, each of which was inserted 20 mm into the sapwood of the tree bole, spaced 4 cm apart (Granier, 1987).

Soil water content was measured using temperature conductive ceramic probes (Campbell Scientific, 290XXX) buried at 10 and 22 cm depths 1 m from the base of individual sampled trees. Meteorological data for each site, including hourly rainfall, air temperature, relative humidity, solar radiation, and ozone concentrations were obtained from ongoing monitoring systems maintained for longer term ecological or air quality studies associated with each of the three sites.

Because the sapflow and soil water data indicated that trees at the Look Rock site were using more water in response to high level ozone exposures, we conducted exploratory analyses of local stream flow data. Our suspicion that increased water use by forest trees would be manifested as a reduction in base flow status attained late in the growing season was substantiated in initial analyses and led us ultimately to collect and analyze streamflow data for the 3 nearest continuously instrumented streamflow gauging stations. Data were obtained for 24 years from Walker Branch Watershed, a 96 ha research watershed maintained by Oak Ridge National Laboratory. In addition USGS data for Cataloochie Creek, NC (a 128 km^2 watershed at the northern edge of GRSM with 22 years of data) and Little River, TN (a 270 km^2 watershed which drains the western slopes of GRSM with 7 years of data) were included. A wide variety of monthly scale meteorological indicators such as the Palmer Drought Severity Index were coupled with indicators of water supply (rainfall and soil moisture status) and demand (temperature, VPD, and sapflow) to evaluate factors influencing stream flow in the August to October time interval.

Statistical Analysis - The automated data systems used in the dendrophysiological analyses provide a very "data rich" environment which affords adequate testing power for both standard multivariate analyses (step-wise regression) as well as more advanced time series statistical approaches. Because of the covariance of many of the variables that influenced ozone formation and plant responses to environmental variables, we used stepwise multiple regressions to evaluate individual and combined responses of biological parameters to environmental parameters. We used STATISTIX (a proprietary statistical package for the biological sciences) to identify statistical significance of both parameters and the models constructed from them. A minimum significance level of 0.05 was selected for these analyses.

Results

Interannual and Intrannual Variations in Environmental Conditions- High variation in both ozone exposure and other associated environmental variables typically occurs both within and between growing seasons in the southeastern US. This variability was important to our efforts to characterize short-term responses of tree growth and physiology to environmental stress. A summary of rainfall, temperature, VPD, Palmer Drought Severity Index, and ozone exposures during the three-year measurement period is included for the Look Rock site in Table 1. Comparable rainfall in 2001 and 2002 was apparent with much higher ozone levels in 2002, combined with a mild, mid-season drought. Ozone levels in 2001 and 2003 were very similar, but rainfall was more abundant in 2003.

Table 1. Environmental conditions at the Look Rock site during three years of studies of growth patterns of mature trees .

Parameter			Environmental Conditions 2001	2002	2003
Rainfall (mm)	D121-180		203	228	315
	D181-240		277	258	340
Temperature (^{O}C)	D122-180				
	July (D181-213)		23.9	25.2	24.4
Vapor Pressure Deficit (VPD)					
	Average12h max.	D134-275	0.79	1.12	0.43
Palmer Drought Severity Index					
	D92-305		0.22	-0.54	4.56
	Monthly Minimum		My (-0.03)	Au (-1.83)	My (4.13)
	July (D181-213)		0.27	-1.11	4.94
Ozone Exposure	Sum 60	ppmh	147	171	89
	CumMaxh-60ppb	ppbh	764	1918	776
	AOT40	ppmh	62.5	78	61.6
	AOT60	ppmh	11.5	24.1	11.7

D=day of the year; My = May; Au = August.

Dramatic differences in patterns of occurrence of high ozone episodes and the influence of intervening lower ozone recovery periods are shown in Figure 1.

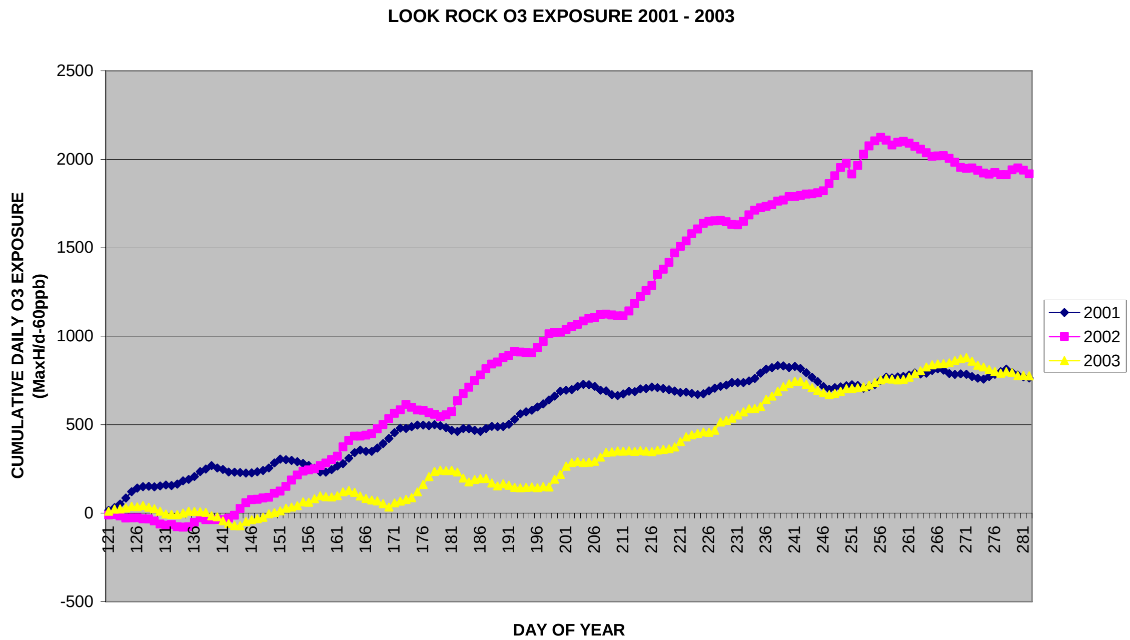

Figure 1. Cumulative ozone exposure during 3 growing seasons indicated that rates of increase in ozone exposure differed widely both within and between years. We have used here a daily running sum of maximum hourly ozone concentration per day-60 *ul*/l. This metric helps one identify periods of potential recovery of vegetation from pollution-induced stress (Yr 2002 >DOY 245) as well as periods within each year when potential ozone stress was increasing rapidly (Yr 2002>DOY 145, DOY>205).

Differences in Seasonal and Annual Growth Rates- Seasonal growth data collected with the manual dendrometers, documented a significant drop in tree growth rates in 2002, compared to 2001 and/or 2003. Red oak data in Figure 2 show that growth rates in 2002 began to drop below those in 2001 and 2003, beginning around DOY (day of year) 165 and differences accelerated in an accumulative fashion thereafter leading ultimately to a growth reduction of about 50%. Thus for this species these curves identify a growth difference and the point at which the tree began to experience stresses that caused a growth reduction in 2002. Data for all other species examined with the exception of chestnut oak (*Q. prinus*) showed a growth reduction of around 25-75% and this was noted at all three sites (Table 2). From a review of the environmental data it was apparent that growth slowdown in 2002 began at a time of comparable rainfall between years and at a time of rapidly increasing exposure of trees to ozone as shown in Figure 1.

Table 2. Differences in annual circumference growth among tree species over three years at three mixed species sites in East Tennessee, USA.

				Annual Circumference Growth		
				% Change from baseline (100)		
Site		**Species**		**2001**	**2002**	**2003**
Look Rock		Yellow-poplar				
	Elev.					
	800m	Bottom site	18	100	-24	-38
		Ridge site	3	100	-42	-42
		Red oak	11	100	-58	13
		Pine	6	100	-48	-2.9
		Hickory	2	100	-14	30
		Chestnut oak	2	100	72	55
				Negative responses		
		N	42		-37	-7.98
				All Responses	-19	2.5
Twin Creeks						
	Elev.					
	700m	Yellow-poplar	9		-62	100
		Red oak	3		-44	100
		Pine	5		-17	100
		Hemlock	3		-22	100
		Sugar maple	3		-64	100
		Red maple	4		-59	100
		Black cherry	7		-76	100
		N	25		-49	
Oak Ridge						
	Elev.					
	300m	Yellow-poplar	12	100	-50	-8

*For these analyses 2001 is used as the baseline year for both Look Rock and Oak Ridge sites, while 2003 represents the baseline ("control") year for Twin Creeks at which measurements were not initiated until 2002. Relative ozone exposures (AOT60) at Look Rock, Twin Creeks, and Oak Ridge were 24.1 PPMH, 12 PPMH, and 18 PPMH respectively in 2002; DOY = day of the year.

By examining the automated dendroband data for 2002 and contrasting growth rates among trees (Figure 2), one can see that there are many periods of increasing and decreasing stem expansion rate when viewed at hourly averaged time scales. The opportunities for associating changes in growth rate with corresponding sets of environmental data within a year goes up dramatically as the dynamics of growth are more clearly resolved.

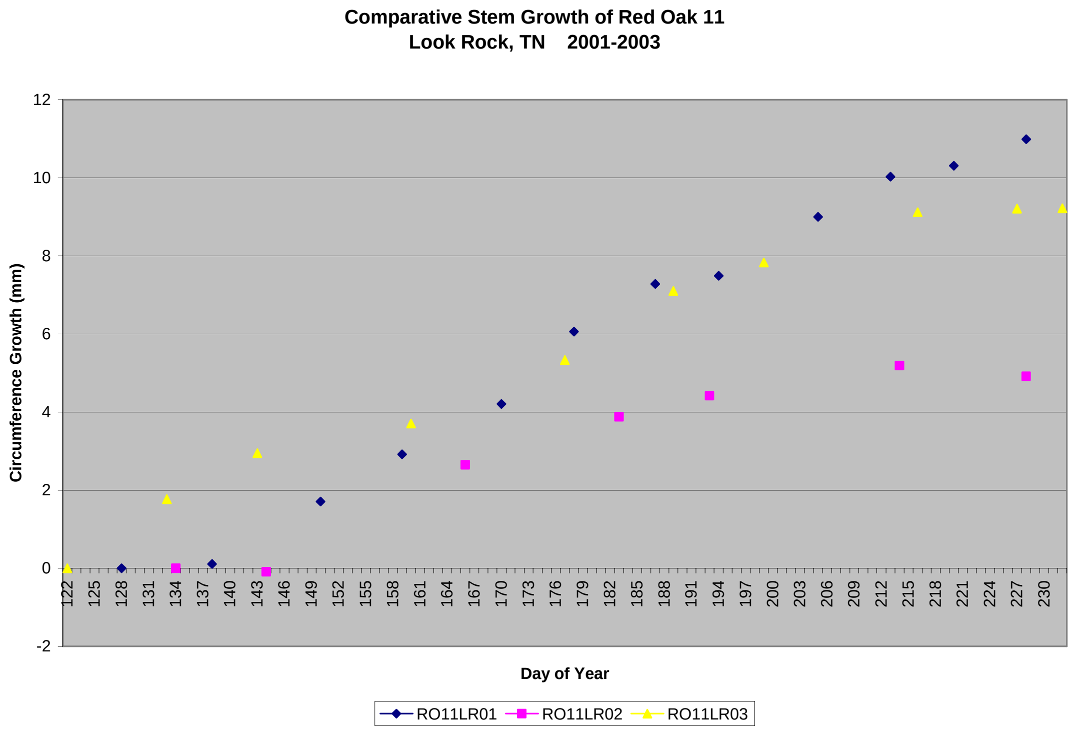

Figure 2. Comparative seasonal patterns of stem increment of Red Oak (RO)11 over three growing seasons at the Look Rock site.

This effect is particularly true of the hourly scale data because one sees that variations in the normal diurnal cycle of stem expansion and contraction during concurrent growth and stem hydration cycles (McLaughlin *et al*., 2003) are amplified for some trees in association with multi-day stress cycles. In Figure 3, we have superimposed the timing of the highest ozone exposure episodes on the seasonal growth curves and one can see amplification of both diurnal cycles and periods of slowdown in stem expansion and/or stem contraction during or immediately following these high ozone episodes. These periods show obvious differences in the mid-day contraction and recovery cycle that reflects the tree's ability to match water demands from sap flow with water uptake by roots.

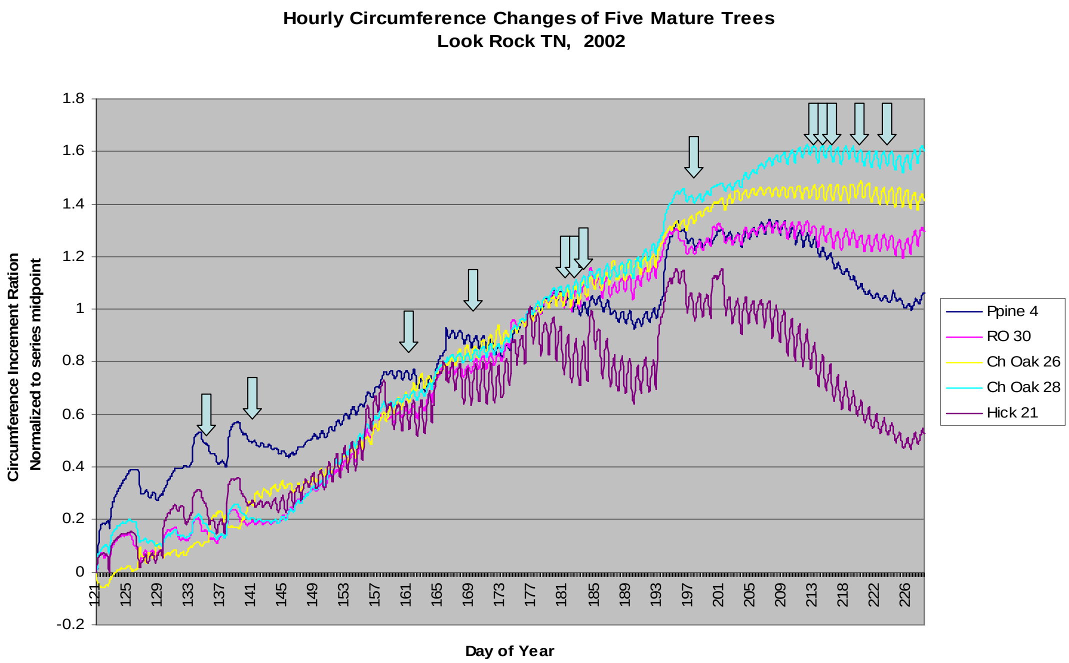

Figure 3. Hourly growth rates of 5 trees from the Look Rock site during the 2002 growing season. The occurrence of days with maximum hourly O_3 exposure levels of $\geq$100ppb for one hour or two consecutive days with $O_3 \geq$ 85ppb are indicated by arrows; Ppine = pitch pine, RO =red oak, Choak = chestnut oak, Hick = hickory.

Modeling Changes in Stem Increment in Response to Environmental Variables-We have approached the challenge of modeling stem increment with two primary objectives. First, we wanted to develop modeling techniques that quantified the specific and relative influences of contributing environmental variables, including ozone, to seasonal growth dynamics. Our second objective was to develop models that would be useful as tools in projecting the effects of changes in ozone on growth of mature forest trees across time and space. The following techniques have been used and are presented in detail in McLaughlin *et al*. (submitted). Stepwise linear regressions were developed with both daily averaged data and hourly data using a wide variety of averaging intervals for both environmental variables and tree increment responses. The multiple regression technique allowed us to examine sequential contributions of each variable after the effects of the variables most strongly correlated with stem increment were considered. A wide variety of environmental and response variables were examined in initial sensitivity analysis. Sensitivity analysis indicated that among the characterizations of ozone exposure, the change in the 3-hour average ozone exposure level beginning 2 hours before the stem increment hour was typically the strongest predictor of stem increment. Inclusion of only times when 3-hour ozone averages were increasing was found to be essential to improved model performance. An hourly-based empirical model for one red oak tree (Red Oak 30, Figure 4) had a predictive R^2 of 0.28 and both ozone (AOT60), and VPD had significant, negative effects on hourly-scale stem increment. Reducing ozone exposure by 50% resulted in an approximate 30% increase in seasonal stem growth, with differences in actual and modeled growth in reduced ozone becoming most apparent after DOY 180. This closely approximated the timing and level of improvement associated with the 50% decrease in ozone exposure (AOT60) that occurred between 2002 and 2001. VPD and rainfall were the strongest predictors, contributing 75% of the R^2 term of the final model. Ozone contributed only about 3%, but was highly significant ($P<0.0001$). Such sensitivity can be attributed to the high statistical power associated with over 2500 data points. By contrast a similar model for Red Oak 11 (data not shown) identified ozone as less significant ($P<0.06$, partial correlation = 3.8%), but for all other trees it was highly significant. However the strength of the influence of VPD and the potential for errors in calculating the true ozone effect led us to test more sophisticated time-series techniques as described in McLaughlin *et al*. (In review).

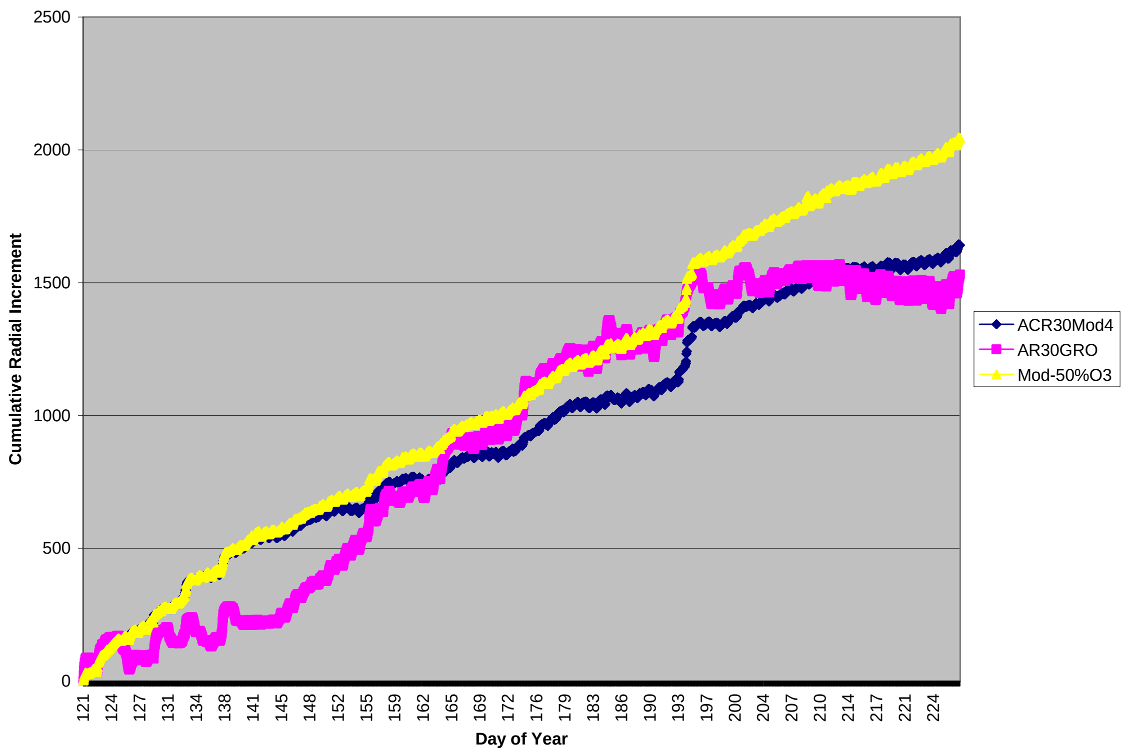

Figure 4. A stepwise multiple regression model of hourly stem increment for Red Oak (RO) 30 during the 2002 growing season growing at Look Rock, identified significant influences of several environmental variables, including hourly changes in 3-hour AOT60 exposure. The model and all included terms were significant at $p\leq 0.05$. Simulated and actual growth under existing conditions are compared to growth with a 50% reduction in high level ozone exposures. ACR30Mod4 = simulated (modeled) accumulated circumference growth, AR30GRO = actual accumulated circumference growth, ACR30Mod4 – O_3 = simulated (modeled) accumulated circumference growth at a reduced ozone level (50%). This is relative growth over the season expressed as accumulative radial increment. The units are electronic units on the sensor and equate to approximately 1.2 mm of actual radial increment over the 124 day measurement interval. The regression model = Model Gro=0.77-0.1098(Chg3hO3AOT60)-0.924(TEMP)-10.47(VPD)-0.00196(Radiation)-0.978(Rain)+1.095(RainHr) R^2=0.277. The model and all included terms were significant at $p\leq 0.05$.

Water Use - Sapflow probes were installed in March 2004 at Twin Creeks, and whole-tree water consumption and diameter growth were monitored throughout the 2004 growing season. Data collection ended the week of 10-25-04. The sample trees were cored and measured in January 2005 to obtain sapwood depth, which is needed to calculate comparable whole-tree fluxes. One tree (TP A2) turned out to have heart-rot, which invalidated the data for that one individual.

Seasonal patterns of sapflow by the trees are illustrated in Figure 5. Each small cycle constitutes one day, ranging from day 208 (July 27) through day 243 (August 31), constituting the main portion of the ozone season that year. Note the complete lack of a seasonal pattern in sapflow. In other words, for most days, sapflow was similar. We could not detect decreases related to high ozone, and in fact, there were essentially no episodes in 2004 (2004 was a near record low ozone year). Tree A3, an asymptomatic tree, had early season rates of sapflow approximately 2X the other trees, but slowed through the season, approaching the sapflow rates for the other trees.

Our initial hypothesis that asymptomatic trees would have lower rates of sapflow due to more closed stomata were not borne out by these data, since the two highest flow rates were in the asymptomatic trees. The three trees with the lowest rates were all symptomatic trees. This suggests that sensitivity in yellow-poplar may not result from greater uptake of ozone because those trees classified as sensitive had the lowest sapflow rates. However, as previously mentioned, 2004 was an unusually wet year with low ozone levels. How the trees would respond under high ozone concentrations and low soil moisture is unknown at this time.

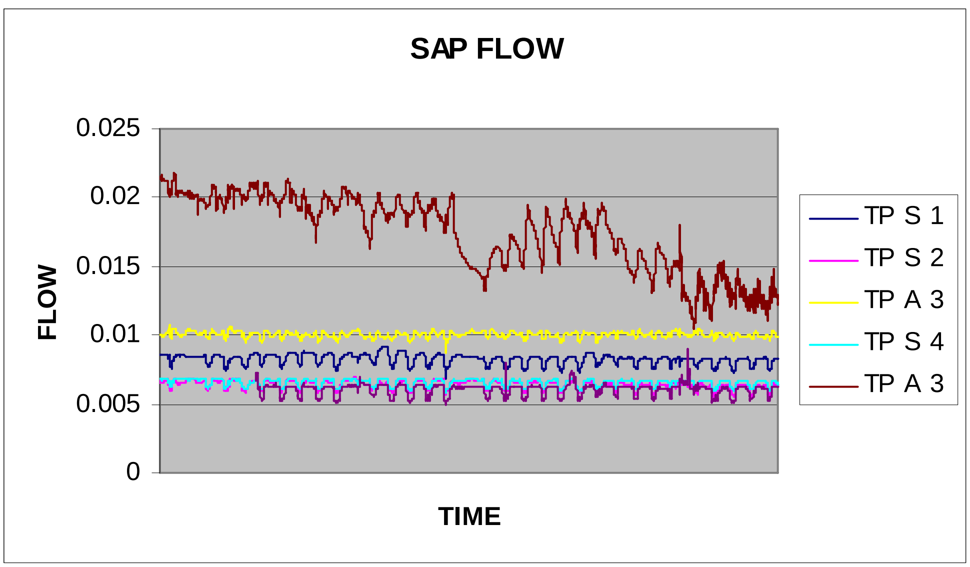

Figure 5. Patterns of transpiration for six yellow-poplar (TP) trees at Twin Creeks for the 2004 growing season. Trees with A in legend are asymptomatic, while trees with S in legend are symptomatic based on Somers *et al*. (1998). Each small dip in each curve represents one day of sapflow. Days run from July 27 to August 31, and cover the main ozone season in GRSM.

Modeling Changes in Water Use – Responses of both soil moisture and sap flow over the 2001 and 2002 growing seasons are summarized for the Look Rock Site in Table 3. These analyses were performed with daily average environmental data. Daily average sapflow and soil moisture at 6 am were the responses measured. Ozone became a highly significant factor contributing to increasing sapflow and decreasing soil moisture in 2002, when ozone levels were high vs 2001 when levels were approximately 50% lower as indicated in Table 3. These data demonstrated that the trees were using more water following high ozone exposures and that soil moisture in the rooting zone of affected trees was measurably depleted by the higher ozone in 2002.

Table 3. Summary of Significant Responses by Predictor Variable Class

Soil Moisture Stress			Sap Flow		
Variable	**2001**	**2002**	**Variable**	**2001**	**2002**
Rain	5	0(7)	Rain	6	1(1)
Temp	1	1(2)	Temp	2(1)	6
VPD	4(1)	10	VPD	3(2)	1(1)
O_3	0	5(5)	O_3	0(3)	4(1)
Sapflow	3	10	Radiation	5	2
			DOY	2(1)	2(1)
Cases	5	10			
			Cases	6	6

* The relative importance of environmental variables in explained responses of trees and soil to higher ozone levels is expressed as the number of comparisons that were significant with $p \leq 0.05$ followed in parentheses by comparisons significant at a $p \leq 0.25$. Numbers of both sapflow sensors and soil moisture probes were increased in 2002; DOY = day of the year.

Based on evidence of soil moisture depletion during high ozone episodes, analyses of the influence of maximum daily ozone exposure on streamflow were conducted for the three nearest gauged watershed systems. Stepwise multiple regression analyses included a wide array of candidate predictor variables. In each case high levels of daily averaged peak ozone exposure were shown to have a highly significant negative effect on baseflow. Our analyses confirmed earlier exploratory analyses and the conclusion that ozone was having a significant adverse effect on baseflow. Averaged stream flow in August through October was best described by a model with the form: [**Baseflow = 52.82 + 0.11099(Precip. August: October) – 0.9376(Avg.Max O_3h/d) + 1.61 (SumO3>60) + 1.359(Palmer Drought Severity Index-August:October)].**

The simulated streamflow matched actual streamflow rather well (R^2=0.764) and model runs with a simulated 25% reduction in ozone levels resulted in significant improvement in baseflow in most years (average +62%) as shown in Figure 6. These analyses of the effects of ozone on forest growth and forest water use provide a spatially and mechanistically coherent picture of significant increases in stress on forest ecosystems induced by anthropogenic ozone. The effects include significant growth reduction of mature forest trees (ca 50%) during high ozone years and increases in water use that likely reduce forest growth during most years. Associated losses in soil water are having significant effects on late season streamflow. Such effects on water availability and on growth of mature forest trees are expected to have many and varied negative implications for forest ecosystem health and function.

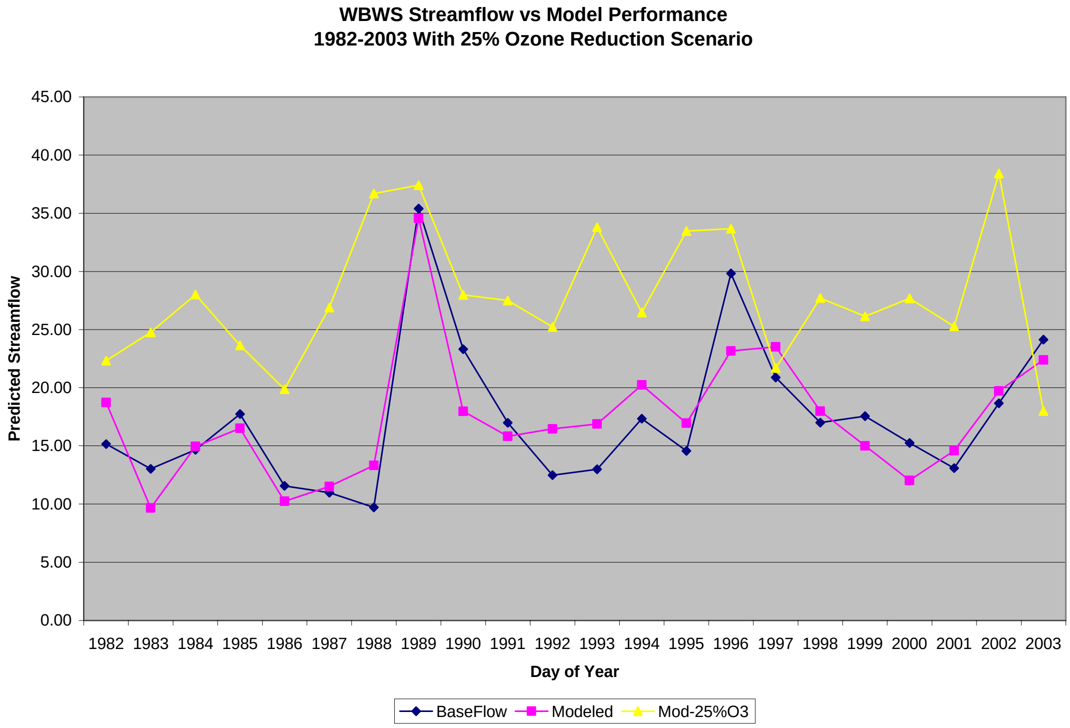

Figure 6. Actual and modeled streamflow at Walker Branch Watershed near Oak Ridge, TN over 22 years. These data represent base flow during the late summer (August through October).

Objective 3. Field Studies of Wildflowers

Methodology

Despite previous studies that have been completed on wildflowers in GRSM (Neufeld *et al*. 1992; Chappelka *et al*. 1997), it is still unknown if ambient levels of ozone pollution affect growth, reproduction or survival of these plants in field situations The previous studies focused on visible foliar symptom expression as an endpoint. To provide information related to the effect of ambient ozone on the growth and physiological response of wildflowers we measured leaf level gas exchange (photosynthesis and stomatal conductance), foliar injury symptoms, and various measures of plant growth (height, number of leaves, etc.) and reproductive capacity (numbers of flowers, fruit, etc.) on cutleaf coneflower (*Rudbeckia laciniata*) and tall milkweed (*Asclepias exaltata*). These species were selected since they have been previously identified as being ozone-sensitive (Neufeld *et al*. 1992; Chappelka *et al*. 1997) and material was readily available in GRSM.

Regarding gas exchange measurements, the most recently fully mature leaf, and an older leaf farther down the stem were measured on several dates for tall milkweed, while every leaf on the flowering stem for symptomatic and asymptomatic cutleaf coneflower were measured at Purchase Knob, in order to determine leaf position/ageing effects. For species that have individuals that differ in sensitivity, approximately five plants representative of each sensitivity class were measured. We attempted to develop relationships between environmental variables, such as light and humidity, and leaf uptake of ozone, to construct models that predict the potential for ambient ozone to cause injury to these species.

At each location (Clingmans Dome and Purchase Knob), total numbers of cutleaf coneflower plants (at least 25 per sensitivity group), injured plants, total number of leaves per plant and ozone-injured leaves were recorded. From these data the average percentage of injured plants and leaves were calculated. Severity of ozone injury was assessed in two ways: 1) percent of foliage injured (number of injured leaves/total number of leaves X 100) and 2) percent of leaf area injured for leaves exhibiting visible foliar symptoms of ozone injury. A modified Horsfall-Barratt rating scale (Horsfall and Barratt, 1945) was used to quantify relative severity of symptoms on injured leaves (classes = 0%, 1-6%, 7-25%, 26-50%, 51-75% and 76-100%). The midpoint of each class was used to calculate average leaf area injured per injured leaf.

Problems of causality arise because there are no ozone-free control sites in the field. At Clingmans Dome, stand characteristics (plant density, height, and location) appear to reduce ozone penetration to cutleaf coneflower leaves in the interior portions of these stands. These plants show little or no ozone injury on the leaves. In the summers of 2003 and 2004, we used cutleaf coneflower plants that exhibited symptoms (near the paved trail) and those that did not (just off the trail, 2 to 4 meters away) to estimate ozone effects in this species. At the Purchase Knob site, we identified symptomatic and asymptomatic individuals within cutleaf coneflower stands and seasonal patterns of ozone injury were documented.

Statistical Analyses -All data were subjected to standard Analysis of Variance (ANOVA), with location, sensitivity class and leaf insertion point (a proxy for leaf age) as fixed variables. For measurements repeated over time (e.g., gas exchange), a repeated measures ANOVA was performed. Foliar injury was analyzed using chi-square analysis. Where appropriate, two-sample, t-tests were conducted.

Results

Stand characterization-During 2003, we monitored the development of coneflowers (*Rudbeckia laciniata* var. *digitata* at Purchase Knob and var. *laciniata* at Clingmans Dome) over the growing season. The summer of 2003 was extremely wet, cloudy, and with low ozone. As a consequence, foliar injury was dramatically reduced this year compared to 2000-2002. Every two weeks, plants in each of 10 1 m^2 plots at Purchase Knob and 5 plots at Clingmans Dome were measured for height, leaf area index (LAI, m^2 leaves per m^2 ground area using a Li-Cor leaf area meter), height of each leaf on the flowering stem, leaf area of each leaf on the flowering stem, and stand density. By mid-June, most stands of coneflower at both sites had reached their peak leaf areas, with LAIs at Clingmans Dome reaching up to 7 m^2/m^2, and slightly lower at Purchase (~5 m^2/m^2). Plants in the understory at Purchase had even lower LAIs of approximately 2.5 m^2/m^2 where the canopy LAI of the overstory trees was also around 2.5 m^2/m^2. Such data were crucial for the development of a stand-level model of ozone uptake and deposition conducted by one of our collaborators, Dr. Pete Finkelstein, U.S. EPA, Raleigh, NC (Finkelstein *et al*. 2004).

Dr. Finkelstein used a high-order closure model of sub-canopy turbulence to estimate ozone profiles in stands of cutleaf coneflower located at Purchase Knob. The model was run for periods coinciding with field studies in 2002-2003, during which we measured vertical concentration profiles of ozone along with measurements of atmospheric turbulence and other meteorological and plant variables. Predictions of ozone profiles by the model were compared with observations throughout the canopy. In general, the model accurately predicted changes in ozone concentrations within the cutleaf coneflower canopy, but over-predicted ozone levels at lower concentrations. This may be due to scavenging of ozone near the ground, but attempts to measure nitrogen oxide (NO) production were unsuccessful due to malfunction of the NO monitor. Further measurements and refinement of the model are needed with this and other species to provide a more accurate prediction of ozone depletion within a canopy of native wildflowers.

Gas exchange- Gas exchange measurements were conducted (2002-2004) on cutleaf coneflower at Purchase Knob, using a Li-Cor 6200 gas exchange system, to evaluate leaf responses to both light and vapor pressure deficits. Plants reached maximum photosynthesis after PAR was approximately 700 umol m^{-2} s^{-1}, or about 32% of full sunlight, and typical of forest understory plants. Photosynthesis of the older leaves on symptomatic plants was reduced more than in asymptomatic plants (Figure 7), but not different for younger leaves with little to no stipple (symptoms). Stomatal conductance peaked at a somewhat lower light level than for photosynthesis, somewhere between 350 and 700 umol m^{-2} s^{-1}, suggesting that stomata were open at low light intensities, and plants were taking up ozone.

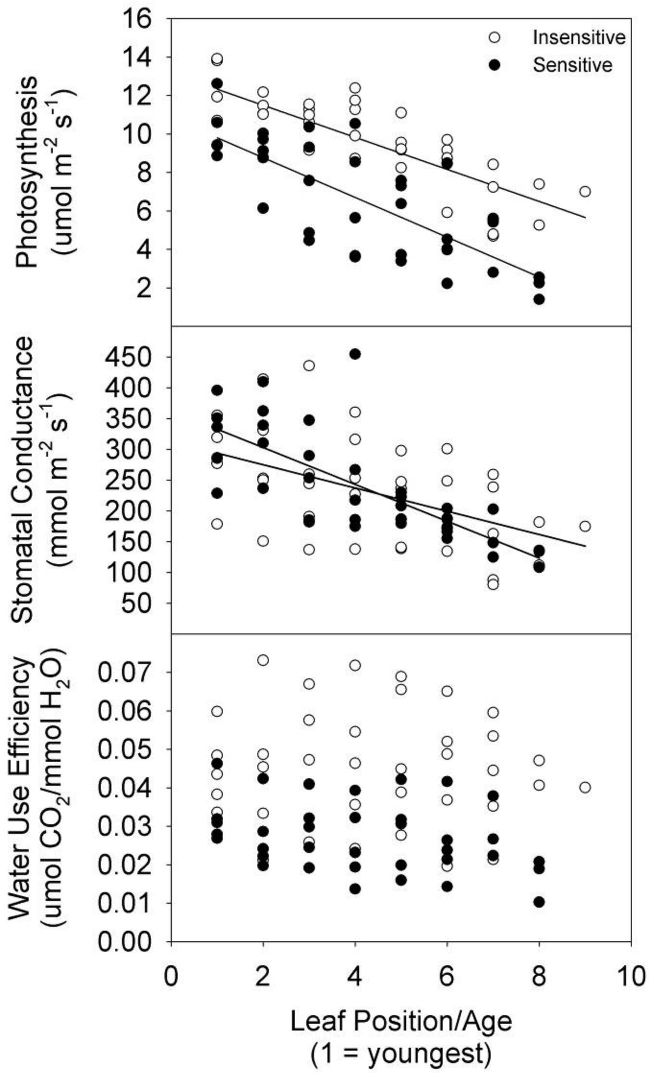

Figure 7. Gas exchange in 2002 at Purchase Knob as a function of leaf position/age in coneflowers.

However, conductance was significantly reduced in leaves with severe foliar injury (> 50%), and these stomata appeared not to be responsive to changes in light; *i.e.*, conductance at both low and high light was approximately the same, whereas for asymptomatic plants, conductance increased with increasing light (Figure 8). These data demonstrate the importance of knowing the stomatal dynamics of a species, and how that is altered by ozone exposure.

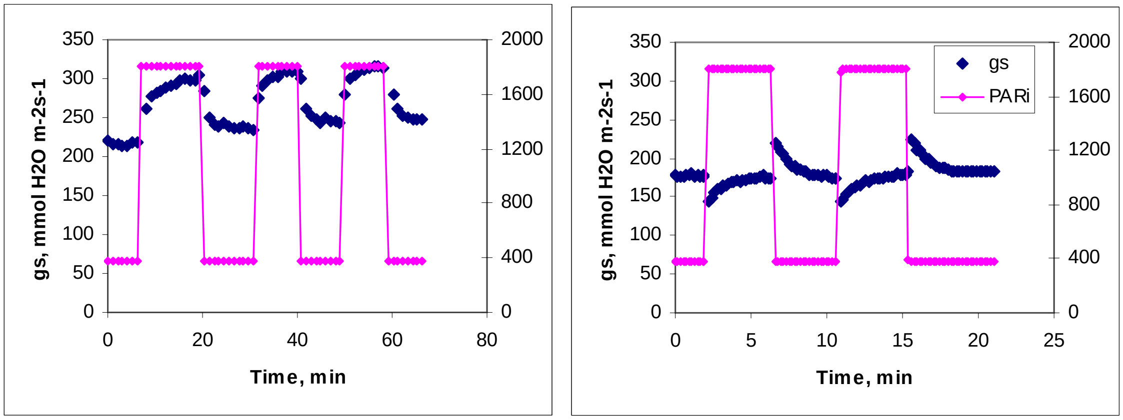

Figure 8. Different forms of response of stomata to sudden changes in light by symptomatic plants (left panel) and asymptomatic plants (right panel). The sudden spikes in the left hand panel are most likely instrument artifacts when changing light suddenly.

Gas exchange measurements were made on plants at Purchase Knob during 2004 using a Li-Cor 6400 gas exchange system (provided by Dr. Nancy Grulke, USDA Forest Service), to evaluate leaf responses to both light and vapor pressure deficits and stomatal sluggishness for cutleaf coneflower. This work was conducted in collaboration with Dr. Grulke. We compared symptomatic and asymptomatic coneflowers for their stomatal responses to changing environmental variables to see if aspects of stomatal conductance (g_s) might help explain the differences in foliar symptom development between symptomatic and asymptomatic individuals. There was no variation in stomatal densities or upper to lower leaf stomatal density ratios that helped to explain differences in ozone sensitivities. Plants of differing ozone sensitivities did exhibit differences in carbon acquisition attributes. Ozone-asymptomatic plants had greater intrinsic transpirational efficiencies, greater maximum assimilation rates under saturating carbon dioxide (CO_2) and light, and greater carboxylation rates than symptomatic plants, and all these factors contributed to a more positive plant carbon balance in asymptomatic plants (Table 4).

Table 4. Steady-state gas exchange characteristics for ozone-symptomatic and ozone-asymptomatic clones of coneflower.

Parameter	**O_3-symptomatic**	**O_3-asymptomatic**	***P* =**
Quantum efficiency (umol CO_2/umol photons)	0.031±0.002	0.048±0.003	0.014
Compensation point (umol/mol)	17±5	3±1	0.046
Carboxylation efficiency (umol CO_2/umol CO_2)	0.017±0.002	0.045±0.006	0.001
P_{max} (umol m^{-2} s^{-1})	12.39±1.39	18.44±2.19	0.046

There was a wide range in stomatal response to changes in both VPD and light, but symptomatic plants as a sub-population were more variable. Stomatal conductance of asymptomatic plants was greater, not lower, than that of symptomatic plants over a broad range of steady-state light levels. Despite greater g_s in asymptomatic plants, there were no statistically significant differences in total leaf water potential or turgor potential between the two ozone sensitivity classes. There was also no difference in timed responses to simulated sunflecks (intermittent light exposure) between symptomatic and asymptomatic plants (Table 5).

Table 5. Summary statistics for stomatal response from low (375 µmol m^{-2} s^{-1}) to high (1800 µmol m^{-2} s^{-1}) light. The time required to reach equilibration at each light level, the average value ($\pm$ 1 S.E.) of stomatal conductance (g_s) at equilibrium, and the instantaneous transpiration efficiency (ITE) at equilibrium are given, in addition to the statistical significance of a two-way analysis of variance (O_3 sensitivity x light level).

Symptom type **O_3-symptomatic**	**Time to equilibrate min**	**gs mmol H_2O $m^{-2}s^{-1}$**	**ITE µmol CO_2/mmol H_2O**
low light	5.0$\pm$0.5	189$\pm$14	3.80$\pm$0.55
high light	5.0$\pm$0.6	213$\pm$22	3.22$\pm$0.53
O_3-asymptomatic			
low light	5.5$\pm$0.7	394$\pm$59	5.64$\pm$0.38
high light	4.8$\pm$0.5	492$\pm$105	6.09$\pm$0.58
	p=	*p*=	*p*=
O_3-symptomatic	0.987	0.027	0.006
light level	0.726	0.047	0.952
O_3 x light	0.828	0.223	0.133

However, g_s of ozone-insensitive plants decreased at lower vapor pressure deficits (VPDs ~0.5 kPa, Figure 9) than did ozone-sensitive plants (>1.5 kPa). Ozone sensitivity appears to be the result of complex individual physiological attributes, varying somewhat independently, that result in expression of the whole-plant response.

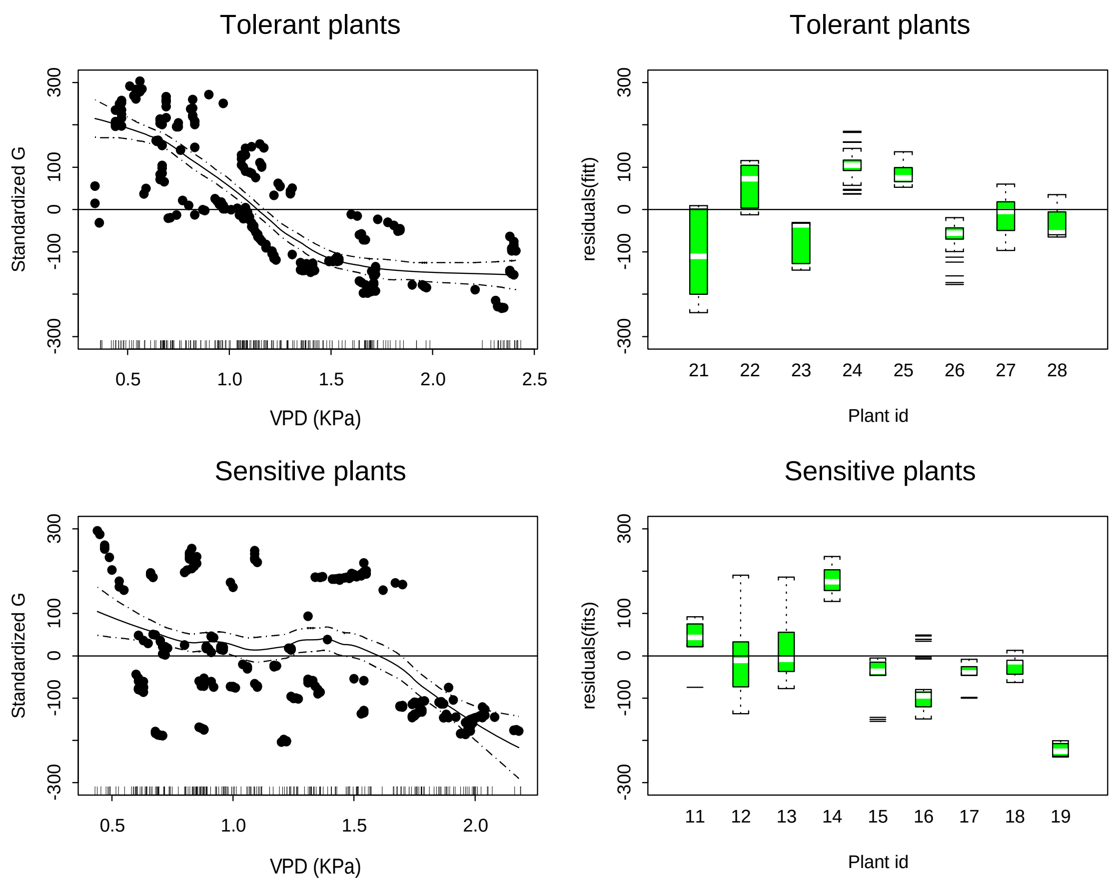

Figure 9. Effect of different light levels on maximum stomatal conductance (*gs*) measured at low vapor pressure deficits (0.5 kPa) observed in O3-sensitive (symptomatic) and ozone–tolerant (asymptomatic) plants. Curves fit using spline techniques. Values for *gs* are standardized to 0 at a VPD of 1.0 KPa. Right hand panels show residuals for graphs on left for each plant measured.

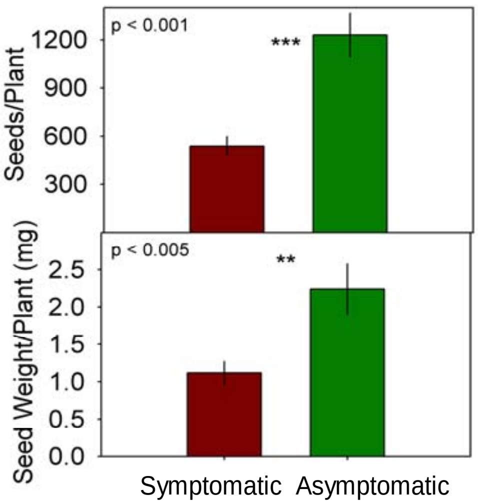

Figure 10. Reproductive effort as seeds per plant (upper panel) and seed biomass per plant (lower panel) for symptomatic (sensitive) and asymptomatic (insensitive) cut-leaf coneflowers at Purchase Knob in 2004. N = 20, differences significant at $p < 0.05$.

Reproductive effort-Seed heads from cutleaf coneflower were collected from 20 symptomatic and asymptomatic plants each at the end of the growing season to determine if reproductive effort is affected by development of foliar injury symptoms. Numbers of flowers, seeds per flower, mean seed weight, and total plant reproductive effort (weight of all seeds per plant) were measured. We also measured plant height and number of leaves. Our data indicate that there were no differences in either height or number of leaves among sensitivity groups, but symptomatic plants produced fewer flower heads per plant, and fewer seeds per flower head than the asymptomatic plants (Figure 10). There were no differences in the mean weights of individual seeds. On a per plant basis, however, symptomatic plants produced 50% fewer seeds than asymptomatic plants. This is a remarkable impact considering the low ozone exposure for year 2004. However, we caution that this is a correlative study only. We can not be certain that sensitive plants have an inherent lower reproductive capacity even when no ozone is present. The only way to confirm these findings would be to conduct a study under controlled conditions, such as in open-top chambers. Nonetheless, the data offer compelling reasons to further investigate the capacity of ozone to lower the reproductive capacity of wildflowers in the field. A second year of measurements was obtained in the summer of 2005, and those data are currently being evaluated.

Ancillary Studies- To provide a better understanding of the complex relationship between ozone and native wildflower populations growing in GRSM a series of ancillary projects (beyond the scope of the original NPS project) were initiated. The majority of these studies were conducted in consort with various collaborators (see below). The results from these efforts are in different stages of completion, but are reported here to provide an understanding of recent efforts in this area of research, and to support the findings from the funded NPS project.

Gas exchange- Dr. Alan Davison, University of Newcastle, conducted numerous measurements on symptomatic and asymptomatic plants during late July 2003 and 2004 to determine if any fluorescence parameters were good predictors of plant responses to ozone. Preliminary analyses suggested that the area above the fluorescence curve, which is a measure of the size of the plastoquinone pool (a part of the electron transport system) is substantially reduced in symptomatic leaves, and highly correlated with maximum rates of photosynthesis. This may offer a simple way of determining the magnitude of photosynthetic impairment due to ozone without resorting to using a more complicated gas exchange system. The data are currently being analyzed in more detail and being prepared for publication.

Biochemistry & Physiology- Measurements were made of the extracellular antioxidant activity in leaves from symptomatic and asymptomatic cutleaf coneflower, crown-beard and tall milkweed in late July 2002-2003 (Dr. Kent Burkey, collaborator). Greater quantities of ascorbic acid in leaf tissue (7-10 μmol g^{-1} fresh weight) were evident in tall milkweed than in either crown-beard (2-4 μmol g^{-1} fresh weight) or cutleaf coneflower (0.5-2 μmol g^{-1} fresh weight). Tall milkweed accumulated ascorbic acid in the leaf apoplast with individuals exhibiting ozone symptoms late in the season having less ascorbic acid than those without symptoms (asymptomatic). In contrast, ascorbic acid was not present in the leaf apoplast of either crown-beard or cutleaf coneflower. Overall, distinct differences in antioxidant metabolism were found in the wildflower species that could differentially affect response to ozone stress. These data will help determine if differences in sensitivity to ozone are related to potential differences in antioxidant activity in plant species (Burkey *et al*., 2006).

Additional data were obtained on the spectral properties of symptomatic vs asymptomatic cutleaf coneflower leaves using a Li-Cor 1800 spectral radiometer. Symptomatic leaves had much lower absorbances than asymptomatic leaves. This response was due to increased reflectance and transmission in these leaves resulting from losses of chlorophyll and possibly by changes in light scattering due to leaf senescence. The production of water-soluble brown pigments in the adaxial leaf surface may reduce photo-oxidative stress in the leaves. Samples of the pigment were sent to Dr. Heinrich Sandermann in Germany and analyzed by Dr. Werner Heller. He found that the pigments consisted of chlorogenic and caffeic acids. It is known that chlorogenic acid is a potent antioxidant (many times more potent than even ascorbic acid).

Chlorophyll levels are often reduced after ozone exposure. Symptomatic leaves of cutleaf coneflower were found to contain lower chlorophyll levels than asymptomatic leaves. A current method used to facilitate the nondestructive measurement of chlorophyll in leaves is the Minolta 502 greenness meter (SPAD meter). Although the meter can be accurately calibrated against actual chlorophyll amounts, we have discovered that this calibration changes in leaves with moderate to severe amounts of ozone injury. Factors associated with the development of leaf symptoms may interfere with the operation of the meter, and a paper describing this was accepted and is in press in the journal *Photosynthesis Research* (Neufeld *et al*., 2006).

Genetics- Preliminary RAPDs (Random Amplified Polymorphic DNA) analyses (Davison *et al*. 2003) indicated the sensitive and less sensitive individuals at Purchase to be genetically distinct, whereas at Clingmans Dome, environment appears to play an important role. To better understand the genetics of ozone sensitivity, leaf samples from symptomatic and asymptomatic plants in 23 different populations in the Park, primarily in the vicinity of Clingmans Dome and Purchase Knob, were collected in late July 2003 and sent off for genetic analyses by Dr. Kirsten Wolff, at the University of Newcastle, U.K. Dr. Wolff has developed a set of markers for this species, and is conducting the genetic analyses. Approximately 15-20 uninjured leaves (upper leaves, sub-tending flowers or flower buds) were collected (one leaf per plant) for each population sampled in 2003. Samples were collected in a random fashion and collected from plants at least 1 meter apart. In addition, 15-25 plants (not necessarily the same plants sampled for DNA fingerprinting), depending upon the size of the population, were observed for evidence of ozone injury during 2003 and 2004. The length and width of each population were measured to estimate the approximate population area. In addition, the latitude, longitude and elevation of each population were recorded using a Magellan Sport Trak Pro GPS unit. It is anticipated that Dr. Wolff will complete the genetic analyses by April 2006, and a publication will then be prepared.

Water relations-An experiment to document the effects of drought on the development of ozone injury in the coneflowers at Purchase Knob was installed in the spring of 2004. The unusually wet and cool weather made that endeavor difficult, but the experiment was conducted from June through September (Melinda Roberts, MS student, ASU). The data are currently being written up by M. Roberts for her thesis. Transpiration rates of symptomatic and asymptomatic plants were monitored, and the data indicated a trend in reduced whole-plant water loss rates in sensitive plants with visible foliar injury compared to asymptomatic plants. These data, collected using a method independent of gas exchange in leaf cuvettes (i.e., using stemflow gages) corroborate the leaf gas exchange results, which show that stomatal conductance in leaves with visible foliar injury are reduced.

Nutritional quality-In cooperation with Dr. Russ Muntifering (Auburn University), leaves from cutleaf coneflower were collected 27-28 July 2004 at three locations in GRSM for a study to examine ozone effects on nutritional quality. Approximately 20 asymptomatic and 20 symptomatic leaves were collected from each population. Both symptomatic and asymptomatic leaves were collected from the mid-canopy and were fully-mature leaves. In addition, 25 plants (not necessarily the same plants sampled for the nutrition study) were observed for evidence of ozone injury. After collection, leaves were placed in a cooler and brought back to the laboratory for analysis. Preliminary analyses indicate that symptomatic leaves contain less nitrogen and less food value to animal herbivores. In addition, it appears that a strong relationship is evident between the production of total phenolics and lignin for the symptomatic leaves, but not as strong for the asymptomatic ones. The implication of these results on animal nutrition is currently being explored in more detail.

Objective 4. Bioindicator Gardens

Methodology

Since elevation has such a marked effect on ozone dynamics and concentration, it is quite possible that plants at high elevations in GRSM are being exposed to greater amounts of ozone, and therefore are at greater risk than low elevation plants. However, elevation is confounded with ozone exposures, so without plants growing at both elevations, it is not possible to test for an ozone effect in and of itself. To get around the confounding factor of elevation, we established common gardens, with a suite of plants known to be sensitive to ozone, at three locations within the Park (Great Smoky Mountains Institute at Tremont, Twin Creeks Natural Resources Center, both of which are low elevation valley sites, and the Purchase Knob Learning Center). However, both the Tremont and Twin Creeks sites were discontinued after the 2003 season. This particular effort was part of the Parks as Classrooms program of the NPS.

Plants of four species were established from cuttings collected within GRSM. The summer of 2002 was the first complete growing season for all the species. The species included in the garden were: tall milkweed, cutleaf coneflower, crown-beard, and a goldenrod spp., *Solidago glomerata*.

Results

The bioindicator gardens were turned over to GRSM personnel during 2003. Purchase Knob Learning Center staff have taken over the routine monitoring and maintenance of the garden at Purchase Knob. These duties include weeding and upkeep, and on occasion, adding new, potential indicator plants. In 2004, for example, *R. laciniata* var. *digitata* (the form found at Purchase Knob) was added to the garden to complement and contrast with the clones that had been established there earlier from Clingmans Dome. Using student volunteers who are trained by Learning Center staff, foliar injury symptoms are monitored on the plants at periodic intervals during the ozone season. Learning Center staff are also responsible for maintaining the passive ozone samplers located at Purchase Knob.

The garden at Purchase Knob has been utilized over the years for several educational events. An educational field trip has been developed for 7th grade and high school students who visit the site to collect data on visible symptoms. Students train in their classrooms using the same website utilized by biological field technicians (http://mona.psu.edu/scripts/FhWeb2.dll/intro). After proving proficiency with the estimation skill, they visit the park to view and rate the percentage of foliar injury symptoms on the plants. Trained Park Rangers then verify the data and post it on-line to an internet database (http://www.handsontheland.org/monitoring/projects/ozone/ozone_bio_search.cfm). This database allows students to compare their data with that collected prior to their visit. They can even view data from previous years. Additionally, root cuttings from plants in this garden have been used to develop gardens in schoolyards throughout the US. As of fall 2005, 62 gardens have been established, mostly in North Carolina and Tennessee. Teachers participating in the study can have their students compare schoolyard data to data collected in GRSM.

Several teacher training seminars have been held over the years in conjunction with partners in the project that include the US Forest Service (Pisgah National Forest) and the GLOBE project. As of fall 2005, 236 teachers have been trained in the ozone biomonitoring garden protocols during 10 different seminars. The GLOBE project recently adopted the Ozone Biomonitoring Garden project as an Advanced Protocol in their Atmosphere Monitoring Study. Learning Center staff, as well as professors from Auburn University and Appalachian State University are working with GLOBE educators and scientists to refine these protocols for the GLOBE program. In cooperation with the federally-funded GLOBE project, new gardens are proposed for additional sites across the country; one is being constructed on the mall in Washington, DC, which will be maintained by the Smithsonian's Museum of Natural History, and another is being established in Philadelphia, PA.

Learning Center staff are working with others in an effort to expand the biomonitoring garden concept to other NPS units. Two presentations on the project have been made to NPS Resource Management and Interpretive staff at meetings sponsored by the Natural Resource Information Division. Additionally, Interpretive staff in the Great Smoky Mountains NP and on the Blue Ridge Parkway have been trained by Learning Center staff in how to recognize and monitor ozone symptoms; this skill has been included in several interpretive programs for the general public. In September 2005, the American Chemical Society highlighted the Ozone Biomonitoring Study in their "ChemMatters" publication for high school students. This magazine has a distribution of over 3 million students (http://www.chemistry.org/portal/resources/ACS/ACSContent/education/curriculum/chemmatters/archive/2005_9_smpissue.pdf).

Publications and Presentations supported by or related to PMIS # 66941

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Acknowledgements

We wish to thank Efrem Robbins, Erik Schilling, William Somers, Lara Souza, Brandon Scarborough and Seth Peoples for assistance in data collection. We would also like to thank Dr. Peter Soule, Appalachian State University, for providing the equipment and laboratory to analyze the tree rings, and Dr. Greg Somers for assistance in data and statistical analyses. Our sincere appreciation goes to Jim Renfro, NPS for coordinating research efforts at the Park.

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