SEASONAL DEVELOPMENT OF OZONE-INDUCED FOLIAR INJURY ON TALL MILKWEED (*ASCLEPIAS EXALTATA*) IN GREAT SMOKY MOUNTAINS NATIONAL PARK

A Thesis

by

Lara Souza

Submitted to the Graduate School

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ABSTRACT

SEASONAL DEVELOPMENT OF OZONE-INDUCED FOLIAR INJURY
ON TALL MILKWEED (*ASCLEPIAS EXALTATA*)
IN GREAT SMOKY MOUNTAINS NATIONAL PARK

(May 2003)

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The southeastern United States, including Great Smoky Mountains National Park (GRSM), experience high ozone concentrations. These concentrations are high enough to cause visible injury on a wide variety of plants in GRSM. One plant that is particularly sensitive to the impacts of ozone is the perennial herb tall milkweed (*Asclepias exaltata*). This species may be a bioindicator for ozone at mid- to high elevations in the Park. However, little is known concerning the seasonal progression of injury on this species, nor the threshold levels of ozone necessary to elicit a response, both of which are necessary to better characterize this species for use as a bioindicator.

The main objectives of my study were to document foliar injury development in tall milkweed at Mt. Sterling Gap, GRSM, throughout an entire growing season on a leaf-by-leaf basis, and to develop ozone exposure

relationships for cohorts of leaves and individual plants, including threshold responses for injury development. An additional objective was to investigate some selected resistance mechanisms that might influence variation in foliar injury among individuals of this species.

I measured foliar injury as percent leaf area injured, and the percent of leaf defoliation during the season. A modified Horsfall-Barratt scale was used to assess the degree of injury. Gas exchange was measured in the field using a Li-Cor 6200. Foliar surveys were conducted from mid-May to August in 2000 and 2001. I classified individual plants as either sensitive or insensitive based on the amount of foliar injury at the end of the first field season.

In the summer of 2001, sensitive and insensitive individuals were placed in small open-top chambers and exposed all season to charcoal-filtered (CF) and non-filtered (NF) air. Gas exchange measurements and foliar injury surveys were conducted two times during the season.

At Mt. Sterling Gap, sensitive plants developed injury earlier in the season and to a greater extent than insensitive plants. Since there were no apparent microclimate differences between sensitive and insensitive plants, this observed variation is most likely genetically based. Both leaf position and age seem to be important factors influencing the seasonal progression of foliar injury within a plant. In general, foliar injury was greater in lower leaves, but only leaf positions 1 and 2 were statistically different from the other leaf positions. When leaves were categorized by their date of origination (by leaf cohort), differences in foliar

injury (stipple) were more apparent. The earlier a leaf cohort was produced, the greater the injury at a given SUM00 exposure index, which suggests that either older leaves were more sensitive than younger ones or older leaves experienced more ozone at a critical period during their development. Foliar injury was greatly reduced in 2001 compared to 2000, possibly due to slightly lower SUM00 and less rainfall, hence less ozone uptake. Leaf positions 1-3 experienced greater leaf loss by the end of August than leaf sets 4-6 and more than half of the leaves that fell off had not shown previous injury consistent with ozone exposure.

No differences in photosynthesis or stomatal conductance between sensitive and insensitive genotypes, either in the field or in the chamber experiment were found during the course of this investigation. There were also no differences in gas exchange between CF and NF grown plants. Therefore, differences in sensitivity within individuals at Mt. Sterling Gap are probably not due to differences in ozone uptake. Apparent quantum efficiency was significantly higher in sensitive individuals compared to insensitive plants in the chamber experiment, suggesting that perhaps sensitive genotypes might have thinner leaves. Antioxidant status was higher earlier in the season than later for both sensitivity types, but insensitive individuals had significantly higher anti-oxidant capacity later in the season, indicating that differential sensitivity may also be a function of leaf biochemical differences.

ACKNOWLEDGMENTS

I would like to thank Drs. Arthur Chappelka, Gary Walker and Ray Williams, all of whom served on my thesis committee. Thanks also go to Dr. Alan Davison for allowing me to use some of his gas exchange data, Dr. Kent Burkey who provided the leaf biochemistry data, Dr. John Ray and Jim Renfro from the National Park Service who funded the ozone passive sampling for the field portion of my project, and Wade Daniels for calibrating the ozone monitors for the open-top chamber experiment. Appreciation is also given to Betsy Cobb and her assistants for help with the greenhouse experiments, and Appalachian State's physical plant workers who aided in the open-top chambers set up. Thanks also go to my numerous field assistants: Seth Peoples, Shay Dumas, Corrie Williams, Efrem Roberts, Karen Geissinger, Jessica Ball, Brandon Scarborough, Angela Griffith, and Tiago Souza. Thanks also go to the Cratis D. Williams Graduate School, the Graduate Student Association Senate, and the National Geographic Society for funding. Appreciation is also given to Dr. Arthur Chappelka for his guidance in training me to assess foliar injury in the field. I would also like to thank Dr. Howard Neufeld, my thesis chair, who was an amazing mentor throughout this whole process and whose invaluable support allowed me to become a better scientist. Finally, I would like to thank my husband, William Farrell, as well as my parents, Rita Duarte Amaral and Jaime Goncalves Souza, who provided moral support and field assistance.

DEDICATION

I would like to dedicate this thesis to my parents, Rita Duarte Amaral and Jaime Goncalves Souza, my husband, William Farrell, and my stepfather, Agenor Silveira for always encouraging and inspiring me.

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INTRODUCTION

The southeastern United States often experiences high ozone concentrations during the summer, despite a relatively low population density and lack of a heavy industrial base found in the northeastern states (McLaughlin & Downing, 1985; U.S. EPA, 1996; Skelly et al., 1997). High concentrations of ozone result from an abundance of precursors such as nitrogen oxides and volatile organic compounds (Krupa & Manning, 1988; Kang et al., 2001), high temperatures, and the prevalence (highest in the country) of stagnant air masses (Mueller, 1994). Additionally, long-range transport, either from the industrialized upper midwest, or the industrialized southwest U.S., brings pollutant precursors into the region (National Research Council, 1991; Dattore et al., 1991; Chameides & Cowling, 1995).

The Great Smoky Mountains National Park (GRSM) suffers from extensive ozone pollution primarily because of its proximity to Knoxville, TN and Atlanta, GA, (which have high levels of ozone in the summer), and from long distance transport from the upper midwest and southwest (Dattore et al., 1991; Mueller, 1994). Although ozone concentrations have declined nationally by approximately 20% over the past 20 years (U.S. EPA, 2001), southern and north-central regions have shown increases in the past decade. Great Smoky Mountains National Park is one of several national parks in which ozone

exposures have significantly increased, nearly doubling between 1990 and 1999 (U.S. EPA, 2001).

Great Smoky Mountains National Park is the most visited national park in the United States (> 9 million visits in 1990, Shaver et al., 1994) and has been designated as an International Biosphere Reserve and World Heritage Site due to the diversity of its flora and fauna. The park is threatened by a variety of biotic and abiotic threats, including exotic, invasive species and air pollution in the form of acidic deposition and gaseous pollutants. Ozone, which is extremely phytotoxic (Krupa, 2000), is the most important gaseous pollutant affecting GRSM (Mueller, 1994).

As part of their mandate as a Class I area, the National Park Service (NPS) is required to investigate and protect resources from any deleterious effects due to a deterioration in air quality (Department of Interior, 1982). Toward this end, the NPS has sponsored air quality effects research in GRSM since 1987. The results of these investigations have shown that over 95 species of plants exhibit putative ozone symptoms in field situations (Neufeld et al., 1992), and symptoms could be reproduced on at least 27 of 39 species exposed to elevated ozone levels in open-top chamber systems. Of these species, the perennial herbaceous plant, tall milkweed (*Asclepias exaltata*), was one of the most sensitive to the effects of ozone. In the open-top chamber experiments of Neufeld et al. (1992), foliar symptoms and premature leaf senescence consistent with ozone exposure were found on tall milkweed plants in every ozone exposure treatment except charcoal-filtered.

Native plants can be useful as bioindicators (Bennett & Stolte, 1985; Manning, 1993; Blum et al., 1997; Manning et al., 2002) or detectors (Manning, 1993) in remote areas where there are no active ozone monitors (Bytnerowicz et al., 1993; Heagle et al. 1995, Chappelka et al. 1997). In GRSM, there are only six active monitoring sites to cover an area of more than 200,000 ha. Therefore, increased knowledge of the responses of bioindicators to ozone will be useful for characterizing ozone exposures in remote areas in the Park, as well as determining the potential impacts ozone may be having on native plants.

The responses of native wildflowers to ozone are much less studied than those of crop plants (Davison & Barnes 1998) but recent studies are showing that ozone can have significant impacts on these plants (Chappelka et al., 1997; Bergmann et al., 1999; Bergweiler & Manning, 1999; Skelly et al., 1999). In GRSM, some of the most sensitive species, in addition to the tall milkweed, include cutleaf coneflower (*Rudbeckia laciniata*), black cherry (*Prunus serotina* Ehrh.), blackberry (*Rubus* spp.), and crownbeard (*Verbesina occidentalis*) (Bennett et al., 1992; Davis & Skelly, 1992; Neufeld et al., 1992; Chappelka & Wergowske, 1993; Chappelka et al., 1997; Chappelka et al., 2003).

Tall milkweeds are particularly suited as bioindicators because of their widespread distribution (at least at higher elevations in the Park), and their hypersensitivity to ozone (Bennett & Stolte, 1985; Neufeld et al., 1992; Chappelka et al.,1997). These plants are common in forest understories and along roadsides in partial shade, and are widely distributed throughout the eastern U.S. In fact, their geographic distribution (USDA Plants Database, 2003)

coincides closely with regions of relatively high ozone in the eastern U.S. (U.S. EPA 1996). Typical populations in GRSM range in size from 25 to over 200 individuals at elevations above 1500 m. Flowering is infrequent in understory populations while those in higher light (e.g., plants growing along the Blue Ridge Parkway) flower much more profusely (pers. obs.). The lack of flowering in the understory is most likely a result of reduced growth caused by low light, since flowering is directly related to plant size (Shannon & Wyatt, 1986). Plants growing in higher light areas are usually larger and more robust (pers. obs). These plants also produce rhizomes and can reproduce vegetatively, but they do not appear to send out runners and form ramets that are inter-connected in large clones.

Although it is known that tall milkweeds are very sensitive to ozone (Neufeld et al., 1992, Chappelka et al., 1997), there is little information available on foliar injury development through time. In addition, we know little about the variation in foliar injury among leaves on individual plants, or the factors responsible for variation in symptom development among individuals. Most studies are conducted only once or twice at the end of a season and assess injury as a function of the seasonal cumulative ozone exposure (Anderson et al., 1988; Heagle et al., 1994; Hildebrand et al., 1996). For example, Chappelka et al. (1997) measured foliar injury two times in mid- to late August. Such data do not allow the determination of threshold exposures necessary to elicit foliar symptoms (Ghosh et al., 1998). Even so, Chappelka et al. (1997) did find that injury progressed rapidly over a short time period in August, with the percentage

of injured leaves increasing from 63 to 79% in just two weeks. They also showed that up to 79% of individuals at Mt. Sterling Gap in GRSM could be classified as sensitive to ozone based on symptom development late in the season, supporting the hypothesis that genetic variation within the population exists in response to ozone. Interestingly, the same study reported that sensitive individuals were significantly taller than insensitive individuals.

In many species, some of this variation in response to ozone exposure likely has a genetic basis (Berrang et al., 1989 & 1991; Reiling & Davison, 1992a; Nebel & Fuher, 1994; Chappelka et al. 1997; Davison & Barnes, 1998; Lee et al., 1999; Elagoz & Manning, 2002; Chappelka et al., 2003; Scebba et al., 2003). In general, sensitive genotypes often have lower ozone thresholds for visible foliar injury and subsequently greater injury when compared to insensitive genotypes (Staedtler & Ziegler, 1993; VanderHeyden et al., 2001). However, the actual mechanisms responsible for variation in ozone sensitivity among and within species have not yet been fully elucidated.

It has been suggested that differences in ozone uptake, leaf anatomy, and biochemistry may all be important factors determining plant responses to ozone (Kollist et al. 2000; Evans et al. 1996; Conklin et al. 1996). Ozone uptake, or dose, is a direct function of stomatal conductance (Bungener et al., 1999; Gruenhage et al., 1999; Zhang et al., 2001; Pasqualini et al., 2002; Schaub et al., *in press*). Ozone can impact stomatal conductance directly by affecting the guard cells or the epidermal cells adjacent to them (Holley et al., 1985; Gunthardt-Goerg et al., 1993), or indirectly by affecting photosynthesis (McKee et

al., 2001; Dalstein et al., 2002; Zheng et al., 2002). As ozone penetrates the leaves, it decomposes and yields free radicals that can damage cell membranes, ultimately causing cell malfunction and/or death (Fryer, 1992). If ozone or its products disrupt the cell membranes of guard or epidermal cells, stomatal conductance can be affected. Recent studies have found stomatal opening and closure to be retarded by ozone-induced turgor pressure loss in the guard cells (Maier-Maercker, 1998; Torsethaugen et al.,1999; Gunthardt-Goerg et al., 2000). On the other hand, ozone can reduce photosynthesis, and feedback from high internal CO_2 can induce subsequent stomatal closure (Calatayud et al., 2002).

Leaf anatomy is also an important factor in the differential plant response to ozone exposure. More sensitive individuals have been found to have thinner leaves when compared to insensitive ones (Bennett et al., 1992; Paakkonen et al.,1997) . Thinner leaves, along with thinner cell walls, allow ozone to penetrate more readily and to damage cells more quickly (Plochl et al., 2000). On the other hand, Ferdinand et al. (2000) found that ozone sensitive genotypes had greater leaf thickness than tolerant ones. What may be more important for facilitating the diffusion of ozone to cells is the amount and/or arrangement of exposed intercellular spaces (Evans et al., 1996; Gravano et al., 2003) rather than just leaf thickness.

Apoplastic antioxidants may also be correlated with ozone resistance in some plant species (Tanaka et al., 1985; Asada, 1992; Burkey, 1999). Antioxidants such as ascorbate are produced intracellularly and then transported to the apoplast where they reduce ozone molecules to non-toxic byproducts.

The oxidized ascorbate is then transported back into the cell where it can become reduced again. During ozone exposure cells tend to produce more antioxidants when compared to filtered controls (Castillo & Greppin, 1988; Luwe et al., 1993; Luwe & Herber, 1995). There is also evidence supporting the idea that sensitive genotypes produce less extracellular ascorbate compared to more insensitive genotypes (Kelly et al., 1995; Dietz, 1997; Lyons et al.,1999; Burkey et al., 2000; Zheng et al., 2000). In other studies, levels of extracellular ascorbate were not correlated with differences in resistance between sensitive and insensitive genotypes in certain crop plants (Burkey & Eason, 2002; Kollist et al., 2000).

My study population was the same group of tall milkweeds at Mt. Sterling Gap, GRSM that was investigated by Chappelka et al. (1997) several years earlier. The goals of my study were to follow foliar injury development in tall milkweed individuals throughout an entire growing season on a leaf-by-leaf basis and, to develop ozone exposure relationships for cohorts of leaves and individual plants, including threshold responses for injury development. By following all leaves from the time prior to any observed injury, until late in the season when many of the leaves had senesced, I could avoid the problem of underestimating foliar injury due to premature leaf loss (Ghosh et al., 1998; Bergweiler & Manning 1999). An additional aim of my study was to investigate some of the resistance mechanisms which might influence the variation in foliar injury between sensitive and insensitive tall milkweed plants. I wanted to determine whether or not gas exchange rates were related to ozone sensitivity, and whether antioxidants,

and in particular apoplastic ascorbate, were correlated with ozone sensitivity.

The studies performed as the basis of this thesis can be divided into two areas. The first of these is presented in Chapter 1, entitled Seasonal Progression of Ozone-Induced Foliar Injury on Tall Milkweed (*Asclepias exaltata*) as a Function of Leaf Position and Age. The second chapter is entitled Possible Causes of Variation in Ozone Sensitivity Among Individuals of Tall Milkweed (*Asclepias exaltata*) in Great Smoky Mountains National Park.

Chapter 1

Seasonal Progression of Ozone-Induced Foliar Injury on Tall Milkweed (*Asclepias exaltata*) as a Function of Leaf Position and Age

(written in the style of, and to be submitted to, the journal New Phytologist)

MATERIALS AND METHODS

Study Site

My study was conducted at Mt. Sterling Gap, GRSM, North Carolina (35^0,42 min, 01 sec, N latitude; 83^0, 05 min, 52 sec, W longitude), at approximately 1,525 m elevation in secondary forest consisting of northern hardwoods mostly. These forests dominate the middle to upper elevations from 1100 -1500 m in GRSM and are characterized by sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula lutea*) (Whittaker, 1956).

Ozone Measurements

Ozone concentrations were measured to develop relationships between ozone exposure and foliar responses. Average ozone concentrations were calculated on a weekly basis for the summer of 2000 and biweekly in 2001 using passive ozone samplers (Ogawa & Co., Inc., Pompano Beach, FL, U.S.A.). These passive samplers collect ozone onto a filter coated with the absorbent sodium nitrite (Krupa *et al*. 2001). In both summers, sampling began in May and continued through early October. Ozone was sampled at 2 m above the forest floor in both years, and additionally at 0.5 m in 2001 at two locations at Mt. Sterling Gap. Passive ozone sampling sites were approximately 75 m apart,

where plants grew in abundance. Reported ozone values for 2001 are the average of these two sites (*see chapter 1 for more details*).

Samplers were retrieved and mailed to the Research Triangle Institute (RTI, NC) for analysis at the end of each sampling period. Filters were extracted and analyzed for the product (nitrate) of the oxidation reaction between ozone and sodium nitrite to obtain the total amount of ozone absorbed. The weekly or biweekly average ozone concentrations (in parts per billion) were multiplied by the number of hours of sampler exposure, and then divided by 1000 to obtain a SUM00 index in parts per million*hours (ppm*hrs).

Foliar Surveys for Ozone-Induced Injury

In late May of 2000 and 2001, tall milkweed individuals were marked with plastic tagging at the base of the stem and numbered accordingly. Ninety-five individuals were randomly selected for foliar surveys at Mt. Sterling Gap for the summers of 2000 and 2001. Surveys were done twice a month from 22 May through 26 August.

Foliar surveys were conducted in the summers of 2000 and 2001 in order to assess the seasonal development of ozone-induced foliar injury. By mid-May plants averaged 2-4 leaf sets (tall milkweed plants have opposite leaves), while by the end of the season most individuals within a population had anywhere from 4-7 leaf sets and ranged in height from 40 to 95 cm.

Plants were measured for growth (height) and flower production throughout the growing season. Light readings were obtained using a Li-Cor 190SA quantum sensor (Li-Cor, Inc., Lincoln, NE) connected to a Li-Cor 6200

photosynthesis system. Photosynthetically active radiation (PAR) was measured on a subsample of plants, consisting of both sensitive and insensitive individuals.

Ozone exposures were measured weekly from June 5 to August 26 in 2000 and biweekly from May 15 to August 7 in 2001. Surveys were ended when most of the sensitive plants had lost a majority of their leaves, but prior to the onset of natural fall senescence, which at this high elevation can begin at the end of August for plants in the understory.

The percent leaf area that was chlorotic, stippled, necrotic, or was missing was evaluated for every leaf. Since necrosis and leaf area missing never amounted for more than 1% of the leaf area, they were not included in further analyses. A modified Horsfall-Barratt scale (Horsfall and Barratt, 1945) was utilized to evaluate percent leaf area injured (0%=1, 1-6%=2, 7-25%=3, 26-50%=4, 51-75%=5, 76-100%=6). Values were averaged for both leaves in a leaf set, and the means used for all further analyses.

Individuals from Mt. Sterling Gap were rated as being either sensitive or insensitive based on the amount of foliar injury, as well as amount of premature leaf loss. Plants with greater than 25% stippling or completely senesced leaves (but only those with a prior history of ozone injury), were classified as sensitive, while those with less than 25% overall injury were classified as insensitive.

The percent of injured plants, percent of injured leaves and percent leaf loss for insensitive and sensitive individuals were calculated for the population as a whole in both 2000 and 2001. At each leaf position, the percent of the leaf

area with stippling and chlorosis across all leaves (injured and noninjured) was calculated for both sensitivity classes. At each leaf position, the percent of the leaf area with stippling and chlorosis was calculated for injured leaves only (in order to assess severity of just injured leaves). Finally, at the end of both growing seasons, the percent leaf loss for each leaf position was evaluated for both sensitivity classes. Abscised leaves were separated according to whether they showed prior instances of ozone-like symptoms or not.

Percent stippling and chlorosis were then plotted as functions of ozone exposure (SUM00 index) by leaf cohorts. A leaf cohort was defined as a set of leaves with a common date of origin. In 2000, many plants already had three leaf sets by the first survey date, while a few had four or even five leaf sets. On the first sampling date (June 5) I assumed that the most recent leaf on each plant had been produced the previous week (May 29). This meant that leaves in this cohort could have come from leaf positions 3 to 5, depending on the plant.

Based on the rate at which the plants added new leaf sets at this time of the year (about seven days per leaf set), an estimate was made of the dates of origin for the next two oldest sets of leaves on all plants. This resulted in an origination date for cohort one of May 16, and for cohort 2 of May 22. For later surveys, the date of origin for a cohort was assumed to be half way between the sampling intervals in which it first appeared. My next sampling date in 2000 was June 20, so cohort 4 was assumed to have originated on June 13. The next sampling date was July 10, so cohort 5 was estimated to have originated on July 3. Only leaf cohorts 1 – 5 were analyzed because few plants produced more than

this number of leaves and statistical analyses were therefore not possible for higher cohorts. A similar protocol was adopted in 2001, but cohort 1 was estimated to have originated the week prior to the installment of the passive samplers. I used the mean ozone exposure for April from a nearby site (Purchase Knob) to get an estimate of the amount of ozone this cohort may have been exposed to during its first week of existence. Dates of origination for cohorts 1-5 in 2001 were April 24, May 1, May 8, May 22 and May 29.

I defined a threshold exposure for injury as that SUM00 exposure where more than 5% of the leaves in a cohort showed level 2 injury or greater. Sometimes the observed injury increased from less than 5% to more than 5% from one survey to the next. When that happened, the threshold SUM00 for inducing injury was the calculated exposure midway between the two sampling times.

Statistical Analysis

Differences among leaf sets for within-population parameters such as percent of leaf area injured, were assessed using analysis of variance. Differences between years for individual leaf positions (or cohorts) were tested using two sample t tests. Prior to analysis, all Horsfall-Barratt ratings were converted to their mean percent injury and analyses were done on the means.

The SUM00 threshhold values for foliar injury were determined as the minimum exposure in which at least 5% of the leaves showed injury. This value was picked to avoid obtaining spuriously low estimations due to injury observations on leaves that occurred early in the season and which were of

questionable origin. Differences among cohorts were tested using ANOVA and compared using Tukey's test (Zar, 1999). Year effects were tested for each leaf cohort using t tests. Finally, a t-test was used to test for any difference in height growth between sensitive and insensitive individuals. Significance for all tests was assumed if $p < 0.05$.

RESULTS

Ozone Exposures

The SUM00 indices were similar for both years (113.6 ppm*hrs and 102.5 ppm*hrs in 2000 and 2001, respectively, Figure 1). Exposures at 0.5 m in 2001 were consistently lower then those at 2 m by approximately 14% (data not shown).

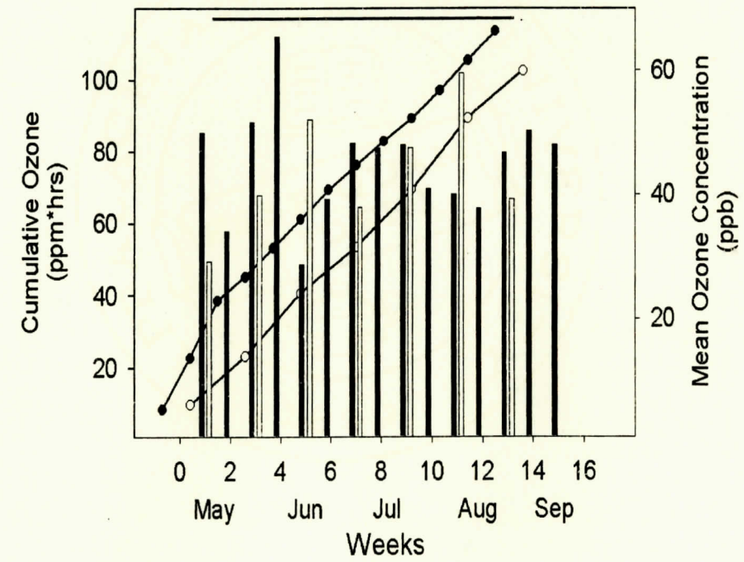

Figure 1. Cumulative ozone (2000, filled circles; 2001, open circles) in ppm*hrs at Mt. Sterling Gap, and weekly (2000, filled bars) and biweekly (2001, open bars) averages of ozone concentration in parts per billion (ppb). Horizontal bar indicates time of year when foliar surveys were conducted.

Rainfall data (Figure 2a & b) from the nearest weather station at Waterville, NC (12 km away, and 1085 m lower in elevation, 35° 46' N, 83° 06' W) indicates that precipitation, averaged across all months, was 12% less in 2001 (70.4 cm) compared to 2000 (79.7 cm). These values are 31% (2000) and 16% (2001) above the long-term normals for that station.

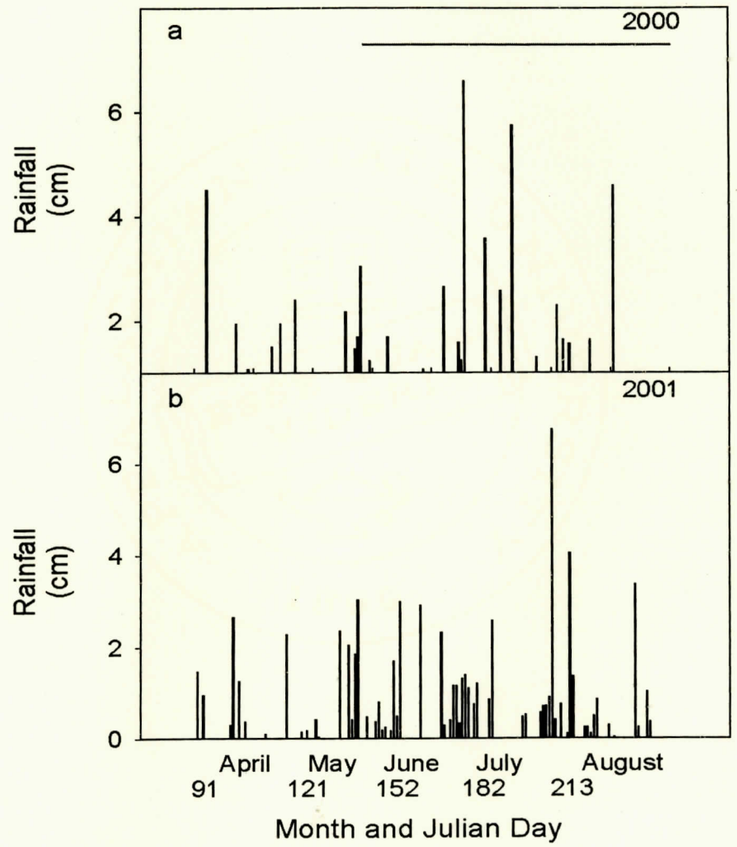

Figure 2. Rainfall data for Waterville, NC for 2000 and 2001. Values are daily precipitation from April to August. Waterville station is 12 km away from Mt. Sterling Gap. Horizontal bar indicates time of year when foliar surveys were conducted.

There were no distinctive periods of drought in either year, with the longest dry period (which occurred once in both years) lasting for nine days. There were more dry periods lasting six or more days in 2001 (8) than in 2000 (3). Temperature trends for mean maximum values did not depart from the long-term normals by more than 5% in either year (National Climatic Data Center, 2003). The portion of the graphs (Figs. 1 & 2) between the arrows indicates the time period during which the foliar surveys were made.

Whole Plant Results

Growth

There were no significant differences in height between sensitive and insensitive individuals at the Mt. Sterling Gap site in either 2000 ($p = 0.667$) or 2001 ($p = 0.096$) (Fig. 3).

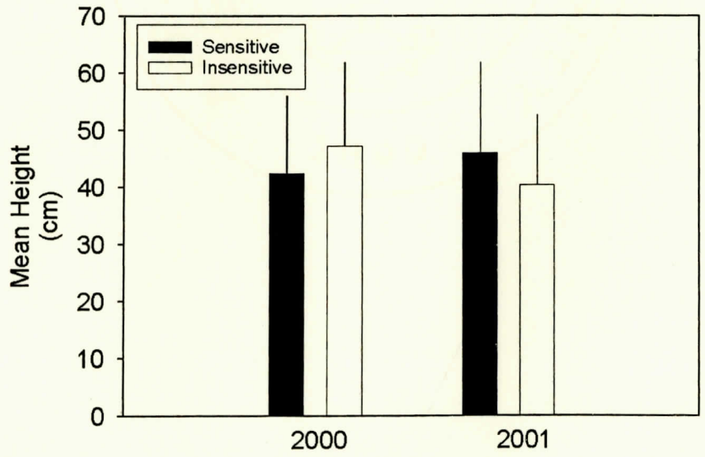

Figure 3. Mean height of sensitive and insensitive tall milkweeds at Mt.Sterling Gap. Values are means ± SE. N=25-70.

Frequency distributions of PAR values for sensitive and insensitive plants (Fig. 4) indicate no major differences, suggesting that sensitivity was not a function of the amount of light received by a plant.

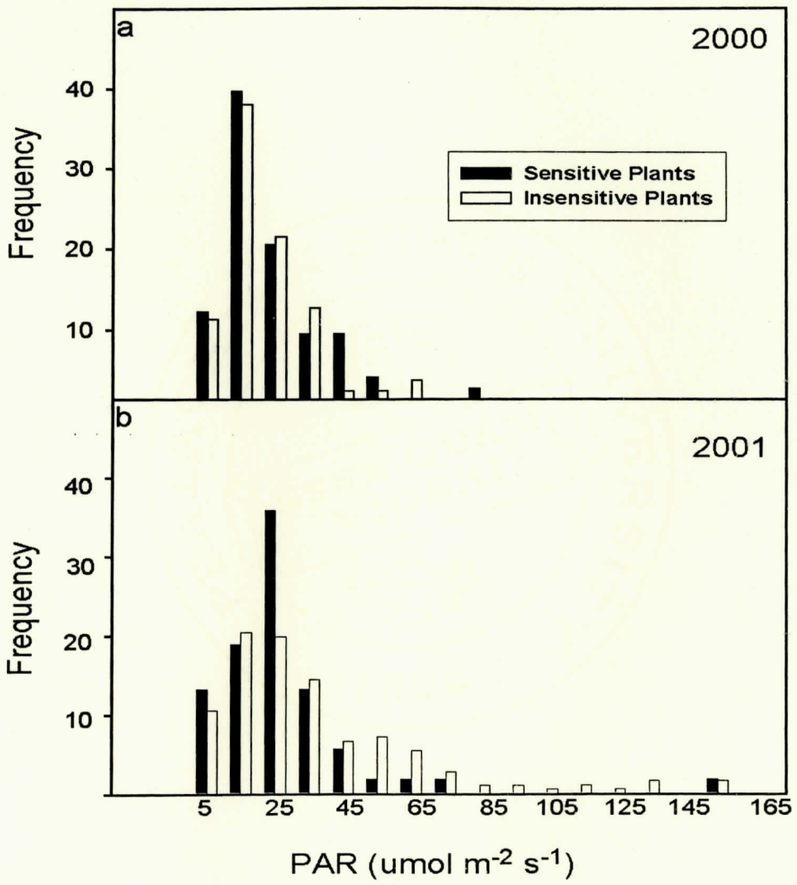

Figure 4. Frequency distribution of PAR for sensitive and insensitive plants at Mt. Sterling Gap, 2001. N = 53-181.

Foliar Injury

After an initial period where no injury was found, the percent of symptomatic sensitive plants increased linearly with time in both years, while increases were more abrupt and closer to the end of the season for insensitive individuals (Fig. 5).

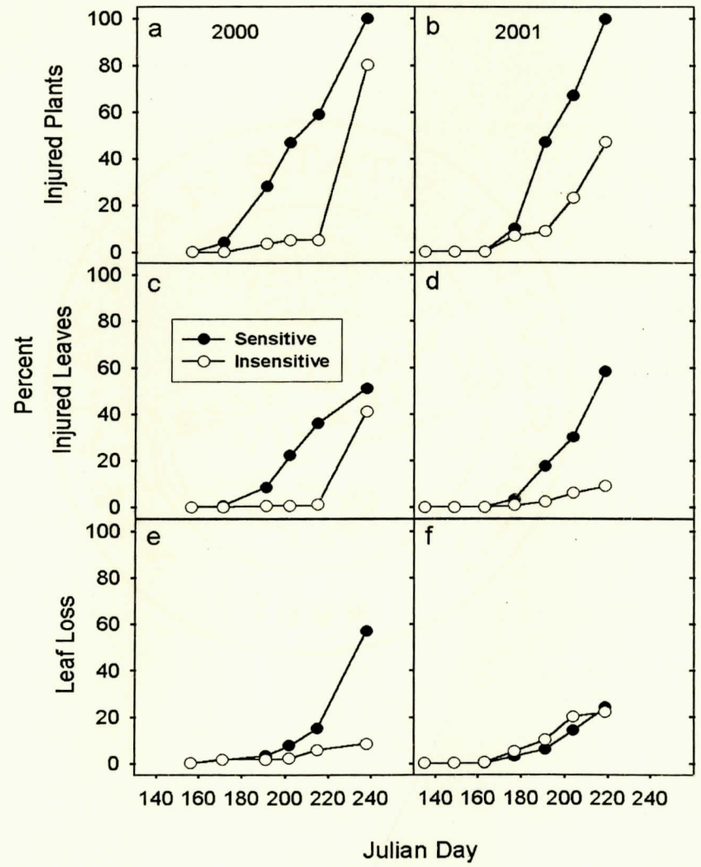

Figure 5. Percent injured plants (a & b), injured leaves (c & d), and leaf senescence (e & f) of sensitive and insensitive individuals vs. Julian day for 2000 and 2001. A total of 95 plants were surveyed each year

Sensitive individuals began showing ozone-induced foliar injury earlier in the season when compared to insensitive individuals (Fig. 5a & b), especially in year 2000. Less than 80% of insensitive individuals in 2000, and 50% in 2001, were symptomatic. The percent of leaves showing injury (Fig 5c & d) followed a similar pattern as that for percent symptomatic individuals, with the exception that insensitive individuals in 2001 had very few symptomatic leaves. The percent of symptomatic leaves for sensitive individuals was similar between years (51% and 58% in 2000 and 2001, respectively) while the percent of injured leaves for insensitive individuals was slightly lower in 2000 (41 %), and much lower in 2001 (9 %).

Percent leaf loss for sensitive individuals was greater in 2000 than in 2001, while the opposite was true for insensitive individuals (Fig 5e & f). As a result, there were no differences in the percent leaf loss between sensitive and insensitive individuals in 2001 (Fig 5f).

Leaf Set Responses

Changes in chlorosis and stippling through time for every leaf set (both symptomatic and asymptomatic leaves included) on both classes of plants are shown in Figures 6 and 7. Sensitive individuals had significantly greater ($p=0.004$) percentage of their leaf area with chlorosis in 2000 than in 2001, whereas percent leaf area chlorotic in insensitive individuals did not differ ($p=0.319$) between years. The opposite was true for percent leaf area stippled where insensitive individuals' leaves were significantly more stippled ($p=0.004$) in 2000 than 2001, while there were no differences ($p=0.131$) between years for

sensitive individuals. By the end of the 2000 season, the oldest leaf set of sensitive individuals had significantly greater chlorosis ($p = 0.007$) and stippling ($p = 0.001$) compared to the other leaf sets (Figs. 6a & 7a).

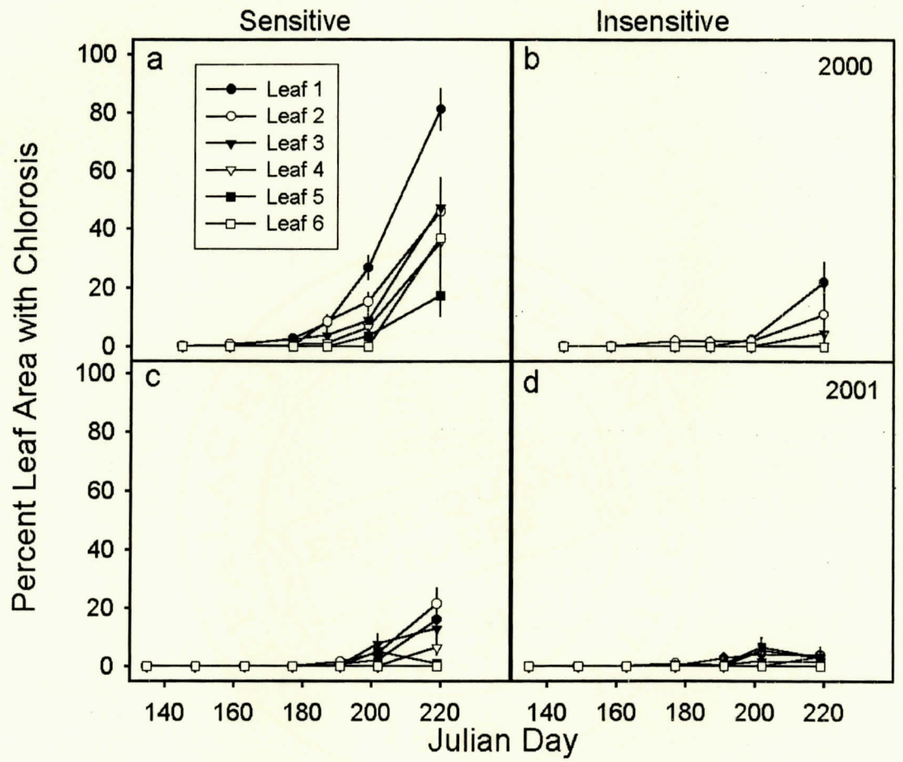

Figure 6. Percent leaf area with chlorosis (for all leaves) of sensitive (a & c) and insensitive (b & d) individuals at each leaf position (Leaf 1 = oldest, Leaf 6 = youngest) vs. Julian day for 2000 and 2001. Values are means ± SE, N=276-368.

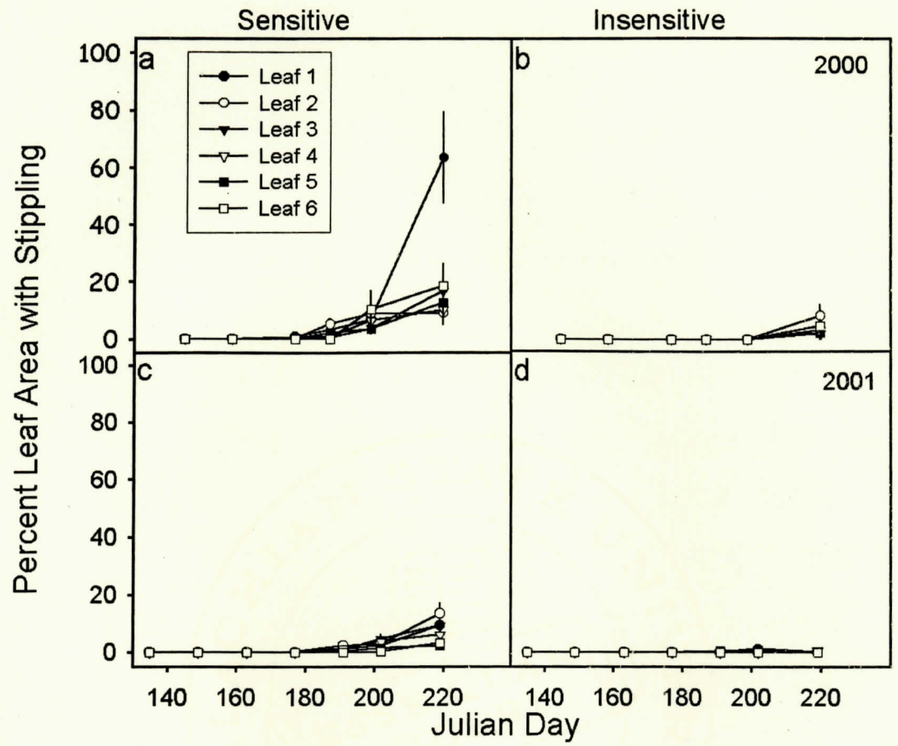

Figure 7. Percent leaf area stippled (for all leaves) of sensitive (a & c) and insensitive (b & d) individuals at each leaf position (Leaf 1 = oldest, Leaf 6 = youngest) vs. Julian day for 2000 and 2001. Values are means ± SE, N=276-368.

There were no differences among the remaining leaf sets. The mean percent chlorotic leaf area was 81% ± 7% SE for leaf set 1 in 2000 while the mean percent leaf area stippled was 63% ± 15% SE (Figs. 6a & 7a). Leaf set 2 had the next highest amount of chlorosis (46% ±11 SE) followed closely by leaf set 3 (47% ± 10% SE).

In 2001, percent injury (chlorosis and stippling) in sensitive individuals was greatly reduced compared to year 2000 (Fig. 6c). Although no differences in chlorosis were observed between leaf sets 1 and 2, leaf set 2, which had slightly higher injury (22% ± 5% SE) was significantly different ($p = 0.002$) from all the younger leaf sets. There were no significant differences among leaf sets for stippling in this year (Fig.7c).

Insensitive individuals (which by my definition could not have had more than 25% leaf area injured) showed no significant differences among leaf sets for chlorosis in 2001(Fig. 6d) and stippling in either 2000 or 2001(Fig. 7b & d). However, older leaves were significantly more chlorotic in 2000 ($p = 0.005$), and the amount of chlorosis much lower compared to year 2000 (Fig. 6b & d).

Foliar injury severity for chlorosis and stippling in injured leaves only is shown in Figures 8 and 9. There was a general trend for higher chlorosis in older leaves for both 2000 and 2001 (Fig.8). The maximum percent leaf area affected was 80% in the oldest leaves in 2000. Leaf positions 1 and 3 were significantly more chlorotic than leaf position 2 ($p=0.005$) in 2000, which was similar to all the other leaf positions. Percent leaf area with chlorosis was similar among leaf positions for year 2001.

The oldest leaves had over half their leaf area stippled (64%) in 2000, and together with leaf set 3, were significantly ($p=0.003$) more stippled than leaf positions 2, 4 and 5 (Fig. 9). In 2001, there was substantially less injury and no differences among leaf positions.

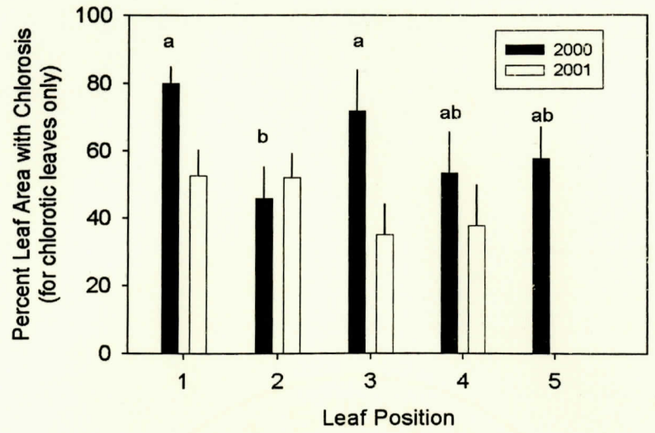

Figure 8. Percent leaf area with chlorosis (symptomatic leaves only) of sensitive individuals vs. leaf position (Leaf 1 =oldest, Leaf 6 =youngest) for 2000 and 2001. Values are means ± SE, N=15-56. Within years, bars with different letters are statistically different ($p < 0.05$). Bars without letters are all the same.

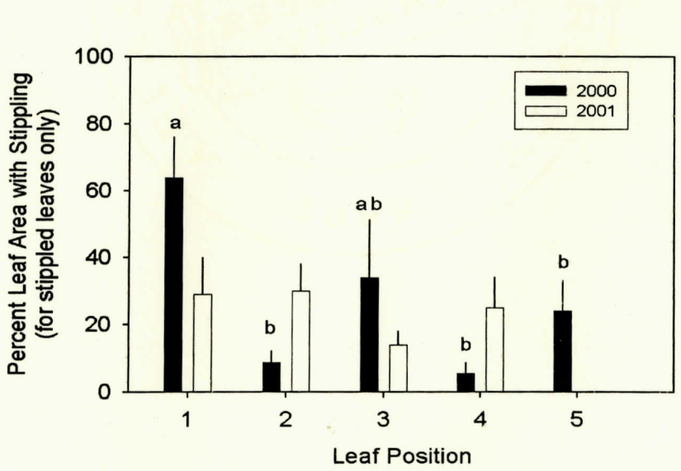

Figure 9. Percent leaf area stippled (symptomatic leaves only) of sensitive individuals vs. leaf position (Leaf 1 = oldest, Leaf 6 = youngest) for 2000 and 2001. Values are means ± SE, N=15-56. Within years, bars with different letters are statistically different ($p < 0.05$). Bars without letters are all the same.

The percent leaf loss of sensitive vs. insensitive plants at the end of 2000 and 2001 by leaf position is presented in Figure 10. Older leaves in both years were lost more frequently than younger leaves in both classes of plants (Fig.10).

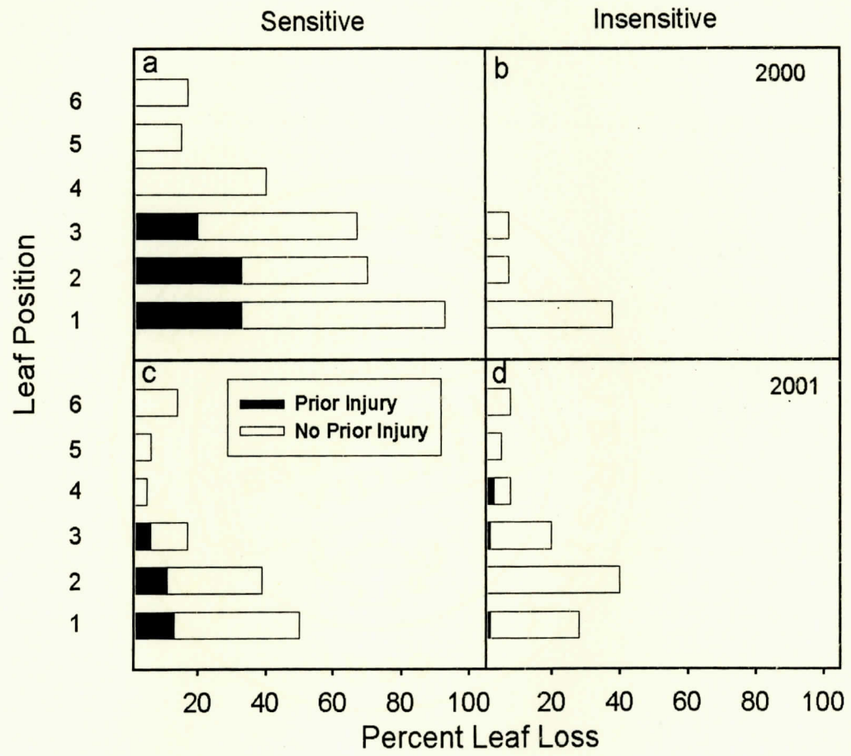

Figure 10. Percent leaf loss of sensitive (a & c) and insensitive (b & d) individuals in August of 2000 and 2001 at each leaf position (Leaf 1 = oldest, Leaf 6 = youngest).

Percent leaf loss was lower for sensitive individuals in 2001 than 2000 (Fig. 10a, and c), while the opposite was true for insensitive individuals (Fig. 10b and d). For leaf sets 1 through 3 in sensitive individuals in 2000, between 20%

and 33% of the leaves that were lost had shown prior injury consistent with ozone exposure such as chlorosis or stippling (Fig. 10a). In 2001, the pattern was the same, but the percent leaf loss was greatly reduced (ranging from 7.5% to 13%, Fig. 10c). The proportion of leaf sets that showed prior ozone injury in insensitive individuals was much lower than that for sensitive plants. Looking at both sensitive and insensitive individuals in 2000 and 2001, more than half of all leaves that were lost never showed any previous injury that could be attributed to ozone exposure.

Relationships Between Cumulative Exposure and Foliar Injury

Foliar injury was greater for leaf cohorts produced early in the season in both 2000 and 2001 (Fig. 11). Among cohorts 1-3, injury was highest in cohort 1 and least in cohort 3 in both years. In 2000, leaf cohort 1 was significantly ($p=0.0001$) more stippled than leaf cohorts 3, 4, and 5. Maximum amounts of injury were similar ($p=0.819$) in both years for cohorts 1-3, in contrast to the large difference in injury between years for sensitive plants when rated by leaf position (Figs. 6 and 7). Cohort 4 showed only slight injury while cohort 5 leaves never showed any foliar symptoms over both years. In 2001, the differences between the cohorts were not as distinct. In fact, in 2001 leaf cohort one was significantly ($p=0.0065$) more injured than leaf cohort 5 only.

The threshold exposures necessary to elicit injury in 2000 ranged, for cohorts 1-3, from 53.3 ppm*hrs to 62.3 ppm*hrs. In 2001 the values ranged from 62.9 ppm*hrs to 66.1 ppm*hrs (Fig. 12). The threshold for cohort 4 was 66.9 ppm*hrs in 2000 and 79.6 ppm*hrs in 2001. There was no threshold for Cohort 5

since, as mentioned above, it did not show any foliar symptoms.

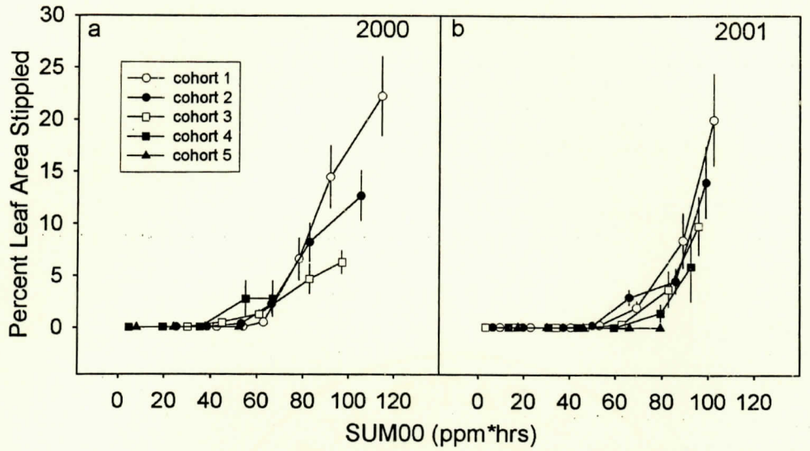

Figure 11. Percent leaf area stippled by leaf cohort (leaves with common origination dates) for sensitive plants vs. SUM00 ozone index for 2000 and 2001. N= 216-392. Values are means ± SE. Cohort 1 = oldest leaves, cohort 6 = youngest leaves).

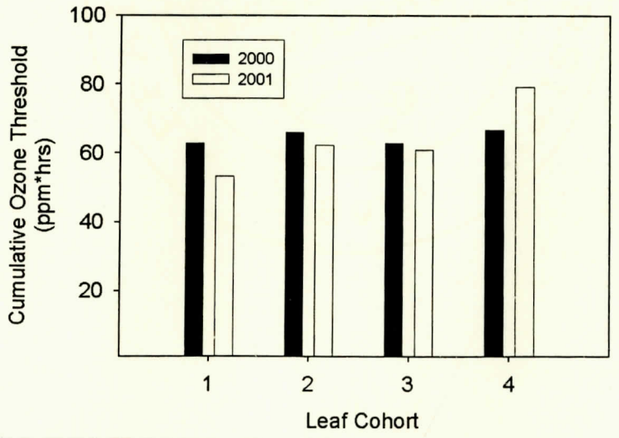

Figure 12. Cumulative ozone threshold (ppm*hrs) vs. leaf cohort for sensitive individuals at Mt. Sterling Gap for 2000 and 2001. Threshold defined as SUM00 where 5% or more individuals in a population showed foliar stippling at level 2 or above. N= 216-392. Cohort 1 = oldest, cohort 4 = youngest.

DISCUSSION

The proportion of *A. exaltata* classified as sensitive in this study (75%) was similar to that found by Chappelka et al. (1997). Results suggest that there has been little selection against the sensitive genotypes over the past seven years at this site. This is not unexpected, given that selection pressures may not be strong enough to eliminate long-lived perennial plants over such a short time period, as has been found with annual plants (Reiling & Davison, 1992b). In fact, given the stochastic nature of ozone exposures at this site, where selection pressures may be high one year and low the next (Berrang et al., 1991), it is likely that these sensitive genotypes are able to persist in the population despite their greater degree of foliar injury. In addition, perennial plants may be buffered against short-term resource limitations caused by ozone by using stored carbohydrates in their rhizomes.

I found no differences in height between sensitivity types in either year of my study (Fig. 3), in contradiction to the findings of Chappelka et al. (1997). This discrepancy might be due to the fact that Chappelka et al. (1997) included individuals from other populations within the Park in their calculations. It also suggests that height may not be a suitable parameter with which to evaluate injury sensitivity in this species.

Foliar injury started earlier, and increased more rapidly for sensitive

individuals as compared to insensitive ones. This correlation between sensitivity and the early appearance of foliar injury has been found by VanderHeyden et al. (2001). However, the study by VanderHeyden et al. (2001), was done in controlled exposure systems. My study is one of the first to document this phenological pattern in the field under ambient ozone exposure conditions. However, when injury was evaluated on the basis of leaf age (cohorts), there were no differences between sensitivity types in the threshold SUM00 necessary to elicit foliar symptoms. This suggests that a knowledge of leaf age is crucial to understanding subsequent symptom development on plants in response to ozone exposure.

Older (basal) leaves showed more injury in both years compared with younger (upper) ones (Figs. 7 & 8), a pattern common to many other studies (Fujinuma et al., 1988; Karlsson et al., 1995; Chappelka et al., 2003), although Paakkonen et al. (1997) found just the opposite for *Betula pendula*. The generally accepted hypothesis is that leaves reach their maximum sensitivity to ozone at about the same time as they reach maturity (Olszyk & Tibbitts, 1981). Changes during leaf ontogeny in stomatal conductance, biochemical defensive mechanisms, and anatomy most likely account for this widely reported pattern. For example, Olszyk & Tibbitts (1981) found that stomata of expanding leaves of *Pisum sativum* closed when exposed to ozone, while fully expanded leaves failed to do the same, resulting in more uptake of ozone by the mature leaves. Tegischer et al. (2002) showed that various anti-oxidants varied according to needle age in *Picea abies*. Finally, as leaves mature, the amount of internal leaf

air spaces tends to increase (James et al., 1999), which may facilitate the diffusion of ozone to the mesophyll cells, thereby resulting in greater injury (Plochl et al., 2000).

Few longitudinal studies have documented the progression of foliar injury on leaves at different positions on a plant throughout a season. Because leaf position and age (i.e., also exposure) are confounded, it is difficult to separate the influence of these two factors on leaf sensitivity to ozone. There could be physiological alterations that arise because of the position of the leaf on the plant (Lee et al., 1999) or changes in the microclimate of particular leaves that affect sensitivity as newer ones are produced. Leaves produced later in the season may experience greater vapor pressure deficits and increased drought stress, both of which may affect their physiological development (Patterson et al. 2000) and sensitivity to ozone (McLaughlin et al., 1982; Balls et al., 1996; Bungener et al., 1999; Ribas et al., 1998).

Ozone-induced foliar injury was more pronounced in 2000 than in 2001. This difference was most likely the result of lower ozone uptake in 2001, and perhaps a slightly lower ozone exposure, although the SUM00 index for 2001 was only 9% less than that in 2000. Rainfall was 12% less in 2001 and there were eight periods with six or more days between rainfall events compared to only three in 2000. Both of these conditions may have resulted in increased in drought stress on the plants. Drought in 2001 could have lowered stomatal conductances and reduced ozone uptake (Kolb & Matyssek, 2001; Reich, 1987), thereby protecting plants from ozone that year (Bungener et al., 1999; Lee et al.,

1999). If true, then if these plants are to be used as bioindicators of ozone exposure, their water status may need to be monitored if year-to-year variations in foliar injury are to be adequately explained.

Although the trend was for foliar injury to increase with the age of the leaf, only the first or second leaf sets actually showed statistically greater amounts of injury than the other leaf sets. This suggests that there could be major impacts of ozone on plant growth belowground, since lower leaves generally send proportionally more photosynthates to the roots than upper leaves. Taylor et al. (2002) showed, for example, that ozone exposures on the perennial plant *Spartina alterniflora* could not be detected by changes in aboveground growth, while root growth was reduced by more than 30%. Therefore, it is possible that in these tall milkweeds root and rhizome growth is being negatively impacted. Future studies should concentrate on the effects of ozone on belowground processes in perennial wildflowers. Reduced root growth, and perhaps lowered carbohydrate concentrations, might eventually lower growth and reproduction (Shannon and Wyatt 1986) in the understory environment where these plants grow, since many understory plants are severely carbon limited because of the lack of light (Neufeld and Young, 2003). Bergweiler & Manning (1999), for example, found impacts on flower production in *Apocynum androsaemifolium* with little or no visible foliar injury.

The severity of injury was greatest in the oldest leaves for chlorosis and stippling (Figs. 8 & 9) in year 2000, but not 2001. In fact, values obtained for injury severity were similar to those of percent leaf area injured across all leaves,

injured and healthy (Figs. 6 & 7). Aside from foliar injury, other parameters should also be measured (such as leaf loss), since the possible interactions between ozone and leaf position among leaf sets other than the oldest may be hard to detect.

In this study, observed levels of leaf loss suggest that leaf senescence may be substantially accelerated in tall milkweed plants growing in the field. More than half of the leaves that fell off had not shown any *a priori* injury consistent with exposure to ozone. Similar patterns of accelerated leaf fall without visible injury have been reported in a wide variety of plant species (Keller, 1988; Reiling & Davison, 1992b; Wiltshire et al., 1993; Bergmann et al., 1995; Braun & Fluckiger, 1995; Pell et al., 1999; Back et al., 1999; Drogoudi & Ashmore, 2000). Evidence that ozone is causing the accelerated rates of leaf fall is supported by the data of Neufeld et al. (1992), showing that potted tall milkweed plants grown in charcoal-filtered chambers did not lose any leaves during the growing season compared to plants that received ozone, grown in either ambient air plots or exposure chambers.

In my study site, most of the tall milkweed leaves had abscised by the end of August or early September, despite relatively mild weather conditions at the end of the season. Premature leaf fall may affect the nutrient status of plants by reducing rates of retranslocation back into the plant (Wiltshire et al., 1993). In addition, early leaf loss may alter nutrient recycling patterns by adding nutrient-rich litter to the forest floor at inappropriate times. Since herbaceous leaf litter decomposes more rapidly than tree leaf litter in general (Muller, 2003), ozone

effects may have the potential to affect nutrient cycling in forest ecosystems. Finally, even though growth impacts from premature leaf fall could be small and difficult to detect, their cumulative impacts may be significant over longer time periods (Wiltshire et al., 1993). More work should be done concerning how ozone affects carbohydrate accumulation along with the role it plays in leaf senescence and leaf fall.

The difference among leaf cohorts for foliar stippling was stronger than when it was plotted versus leaf position (Fig. 11), reflecting the observation that cohorts were related by date of origin, and hence exposure, whereas leaf position did not always correspond to the same exposures across plants. Despite the differences in the amount of foliar injury between years, there was a consistent pattern whereby leaf cohorts that originated earlier in the season showed higher injury than later cohorts at the same cumulative ozone exposure. For example, leaf cohort 1 had over 20% stipple, while leaf cohort 2 only had approximately 11%, and cohort 3 just 6% at a SUM00 of 102.5 pppm*hrs (Fig. 8). Some of this difference might be the result of the temporal distribution of ozone episodes during the season. For example, in 2000, there was higher ozone in the first four weeks of the season compared to later and this might have contributed to the increased injury on leaves produced early in the season. The greatly reduced amount of stipple in cohort 4 and complete lack in cohort 5 is more difficult to explain, but may be due to higher temperatures and increased vapor pressure deficits later in the season, which could contribute to a reduced uptake of ozone by these leaves. Alternatively, leaves that are produced late in

the season may be inherently less sensitive to ozone than those produced early on.

The three oldest cohorts had similar thresholds for foliar injury, while the 4th cohort appeared to require a greater SUM00. This can be explained in part by the wetter conditions in the early portion of the growing season (*see chapter 2*), higher stomatal conductances, and thereby greater ozone uptake. It might also be related to reductions in the anti-oxidant capacity of the leaves through the season, whereby older cohorts become more susceptible as they age. Younger cohorts may not reach an age at which the anti-oxidant capacity declines enough to allow foliar injury to take place.

Conclusions

The population of tall milkweed I studied showed individual variation in susceptibility to ozone exposure. Since there were no apparent microclimate differences between sensitive and insensitive individuals, this suggests that the observed variation is likely genetically based. Because data for mine and a previous study show that the proportion of sensitive to insensitive individuals remained nearly the same over a period of seven years, genetic selection against the sensitive genotypes probably is weak or non-existent.

Both leaf age and position seem to be important factors influencing the seasonal progression of foliar injury within a plant. Injury was generally greater in lower leaves, but not statistically different among leaf positions except for leaf sets 1 and 2. But when injury was categorized by date of origination, differences were more apparent, with older leaves showing more injury. The earlier a leaf is

produced, the greater the injury at a given SUM00, suggesting that either older leaves may be more sensitive to ozone than younger ones, or older leaves experienced more ozone during a critical period in their development. Injury was greatly reduced in 2001 compared to 2000, perhaps the result of a slightly lower SUM00 that year, less rainfall, and possibly reduced uptake. Ozone appeared to cause premature leaf senescence, especially in 2000, and more than half the leaves that fell had not shown typical ozone injury symptoms. The impacts of foliar injury on growth, particularly belowground, and on reproduction remain, to be ascertained.

Chapter 2

Possible Causes of Variation in Ozone Sensitivity Among Individuals of Tall milkweed (*Asclepias exaltata*) in Great Smoky Mountains National Park
(written in the style of, and to be submitted to, the journal New Phytologist)

MATERIALS & METHODS

Field

Study Site

My study site was Mt. Sterling Gap, North Carolina (35^0,42 min, 01 sec, N latitude; 83^0, 05 min, 52 sec, W longitude) in GRSM, located at approximately 1,525 m elevation in secondary forest consisting of mostly northern hardwoods. These forests dominate the middle to upper elevations from 1100 -1500 m in GRSM and are characterized by sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula lutea*) (Whittaker, 1956).

Ozone Measurements

Ozone concentrations were measured in order to develop relationships between ozone exposure and foliar responses. Average concentrations were measured on a weekly basis for the summer of 2000 and biweeekly in 2001 using passive ozone samplers (Ogawa & Co., Inc., Pompano Beach, FL, U.S.A.). In both summers, sampling began in early May (first week) and continued through early October. Ozone was sampled at 2 m above the forest floor in both years, and additionally at 0.5 m in 2001 at two locations at Mt. Sterling Gap located about 75 m apart where plants grew in abundance. The ozone values reported are the average of these two sites (*see chapter 1 for more details*).

The weekly or biweekly average ozone concentrations (in parts per billion) were multiplied by the number of hours of sampler exposure and then divided by 1000 to obtain a SUM00 index in parts per million*hours (ppm*hrs).

Gas Exchange

Gas exchange measurements were performed on plants at Mt.Sterling Gap using a Li-Cor 6200 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE, U.S.A.) to assess temporal and seasonal patterns of photosynthetic rates and stomatal conductances for the 2000 and 2001 growing seasons. The system was calibrated using a secondary CO_2 standard traceable to a National Institute of Standards and Technology standard tank at the Duke University Phytotron. In 2000, the relative humidity sensor malfunctioned on the Li-Cor, so representative values for stomatal conductances were obtained using a Delta T Porometer (Delta T Devices, UK). Later cross calibration studies showed the two instruments yielded similar values for conductance (data not shown). Photosynthetic rates were measured on a monthly basis for one population (Mt. Sterling Gap) from June 12-August 26 2000. In summer 2001, diurnal measurements were taken biweekly with the exception of July, where only one measurement was taken due to inclement weather. Readings were usually taken four times throughout the day (from approximately 0900 until 1400 hrs), although bad weather often resulted in fewer measurements.

Biochemistry

The procedure for extracellular (apoplastic) ascorbic acid extraction was obtained from Burkey et al. (2001) and is shown below. Ascorbic acid can be

found in two different forms. One form is the reduced state (AA), whereas dehydroascorbic acid (DHA) is the oxidized form.

Extracellular ascorbic acid isolation and leaf tissue harvest

Mid-veins were removed from selected leaves and the fresh weights measured. Leaf tissue was vacuum infiltrated with 100 mM KCl and the intercellular wash fluid (IWF), containing extracellular AA, was recovered by centrifugation into an aliquot of 2% (w/v) meta-phosphoric acid and 2mM EDTA (Burkey, 1999). Following IWF recovery, leaf tissue was re-weighed, frozen in liquid nitrogen, transported to the lab under dry ice, and stored at -80°C prior to analysis of AA content.

Tests showed that ascorbic acid in the IWF was stable under these conditions. The presence of glucose 6-phosphate (G6P) was used as a marker for cytoplasm contamination (Burkey, 1999). If a G6P signal was observed, the individual IWF sample was not included in the data set.

Tissue extraction protocol

Frozen leaf tissue was ground in liquid nitrogen using a mortar and pestle, then extracted with cold 6% (w/v) meta-phosphoric acid, and 0.2 mM diethylenetriaminepentaacetic acid. The extraction buffer was prepared fresh each day and used in a ratio of 10 ml g^{-1}FW. The homogenate was centrifuged at 10500 g for 10 min at 4°C. Extract supernatants were assayed for AA and DHA. Recovery experiments using spiked AA showed that AA was efficiently extracted without changes in redox state with this protocol.

Assay of AA and DHA

The AA and DHA present in IWF and leaf tissue extracts were determined independently by monitoring changes in absorbance at 265 nm induced by commercially available ascorbate oxidase and dithiothreitol, respectively (Luwe and Heber 1995). Ascorbate redox status was expressed as the AA/[AA+DHA] ratio.

For the calculation of apoplastic ascorbate content, measurements of leaf weight before and after infiltration with 100 mM KCl and again following the IWF centrifugation step were used to calculate the recovery of the infiltrated solution. The percent recovery for each leaf was used in the calculation such that the reported values are normalized to reflect 100% recovery.

Greenhouse Experiments

Chambers

On 27 August 2000, 100 rhizomes from two populations in GRSM were collected, 50 previously rated as sensitive and 50 as insensitive relative to ozone injury (*see chapter 1*). The rhizomes were transported to the greenhouse at Appalachian State University and transplanted into 3.2 L PVC tubes filled with Metro-Mix 360 soil (Scotts-Sierra Horticultural Products Company, Marysville, OH, U.S.A.). On 12 October 2000, the rhizomes were placed outside where they entered dormancy for the winter. The pots were covered with an insulation material and the soil kept moist throughout the winter. On 22 April 2001, the insulation was removed and all seedlings were exposed to ambient air under 92% shade cloth (Mize Farm and Garden Supply, Inc., Johnson City, TN,

Seedlings were then moved on 6 May beneath 74% shade cloth (PAR = 520 umol m^{-2} sec^{-1}). This light level was similar to the mean maximum light intensity reaching the lower canopy in GRSM. Light intensities were measured with the quantum sensor on the Li-Cor 6200.

Eight small open-top chambers (1.21 m height by 1.04 m in diameter) with clear PVC walls were used to expose plants to either charcoal-filtered (CF) or non-filtered (NF) air. A sheet of plexiglass with a hole of radius 41 cm was placed on the top of the chambers in order to minimize air intrusions from the top. Ozone concentrations were collected from the chambers through teflon tubes connected to either a TECO Model 49 ozone analyzer (Thermo Environmental Instruments, Inc., Franklin, MA, U.S.A.) or a Monitor Lab 8810 ozone analyzer (2B Technologies , Inc., Golden, CO, U.S.A.) . Ozone monitors were calibrated in March 2001 by the North Carolina Department of Environment and Natural Resources. One representative NF and one CF chamber were continuously monitored 24 hrs/day throughout the course of the experiment using a Campbell 21x datalogger (Campbell Scientific, Inc., Logan, UT, U.S.A.). Once-a-week for one hour, ozone in the other chambers was checked to verify that the concentrations were similar. Concentrations were always within 5% of each other. On average, the CF chambers had ozone concentrations about 30 ppb lower than the NF chambers. On 1 June 2001, four sensitive seedlings and four insensitive seedlings were placed inside each open top chamber. Four chambers received NF air, while four others received CF air.

The fertilizer Osmocote Plus (15-9-12) was applied once on 2 April 2001 (Scotts-Sierra Horticultural Products Company, Marysville, OH, U.S.A.). In addition, Enstar II (Wellmark International, Bensenville, IL, U.S.A.), which is an aphid growth regulator, was applied on 3, 17, and 25 July 2001, as well as 8 and 12 September 2001. Another control method used was avid, a miticide (Novartis Corporation, New York, NY, U.S.A.), on 3, 17, and 26 July 2001. A systemic fungicide (Turf and Ornamental Systemic Fungicide, Dayton, NJ, U.S.A.) was applied twice on 18 and 26 July 2001. Although fungicides can be antioxidants, treatments were applied late in July when foliar injury is normally well developed in these plants. Since there was no injury at the time of application, it is unlikely this late fungicide application prevented foliar ozone injury development. In addition, foliar injury at GRSM that year was very low compared to the previous year (*see chapter 1*).

Foliar Survey

Foliar surveys *(see chapter 1 for details of methodology)* were performed in order to assess the development of ozone-induced foliar injury throughout the growing season. Surveys were done on a weekly basis beginning on 22 May and continued through 18 October 2001 in the same manner as they were conducted in the field.

Gas Exchange

Each month, one sensitive, and one insensitive plant, were randomly selected from each chamber for gas exchange analysis. Gas exchange measurements were taken monthly outside the chambers beneath the shade

cloth using the Li-Cor 6200 every two hours from 8 am until 6 pm. In August 2001, gas exchange was measured on an upper (10^{th} leaf from the base) and lower leaf (3^{rd} from the base) as well. In addition to these diurnal measurements, light response curves were performed twice in a classroom inside the greenhouse (July 4-12 and August 11-14). Measurements were made on the third leaf from the base (fully formed) between the hours of 8 am and 2 pm. Light intensities were: 0, 39, 81, 120, 300, 540, 877 and 1400 umol m^{-2} sec^{-1} and the order of measurement was from highest to lowest PAR. Leaves were allowed to acclimate for 30 minutes prior to being measured for gas exchange. Leaf temperatures ranged from 22-28 ^{0}C and relative humidity from 38-69 %.

Belowground biomass

Because plants were needed for another experiment, destructive harvesting for total biomass was not performed. Instead, belowground biomass (fresh weight basis) was compared between sensitive and insensitive individuals in the CF and NF treatments. Rhizomes were removed from the pots in October 2001 and rinsed over a 2.5 mesh size screen. Fine roots growing laterally from the rhizome were removed in order to obtain the new growth from the 2001 season. Both rhizomes and fine roots were allowed to dry at room temperature for two hours and then weighed to the nearest 0.01 gram using a Mettler Toledo PG5002-5 balance (Mettler Toledo, Columbus, OH).

Statistical Analysis

All data were analyzed using SAS version 8.2 (SAS, Inc., Cary, North Carolina, USA). Significance for all statistical tests was assumed for $p < 0.05$.

Gas Exchange

Repeated measures analysis of variance was conducted on photosynthetic rate, stomatal conductance and water use efficiency between sensitive and insensitive individuals for the field and greenhouse experiments. Because ambient light levels in the field varied from plant to plant, linear regressions of photosynthesis and stomatal conductance on light were performed to determine if there were any significant differences between upper and lower leaves on a plant for sensitive and insensitive genotypes. Slopes were compared using a t-test (Zar, 1999). Since we found no differences due to leaf position, upper and lower leaves on a plant were averaged together prior to performing an ANOVA to test for differences in sensitivity. In the greenhouse experiments, a two-way ANOVA was conducted with sensitivity and ozone treatment (CF or NF) as main effects. Post-hoc tests for both the field and greenhouse were done using Tukey's test.

Belowground biomass

A covariate analysis of variance was conducted to detect statistical differences between sensitive and insensitive rhizomes in charcoal filtered chambers and nonfiltered chambers. Rhizome weight was used as a covariate to adjust for potential differences in size at the beginning of the experiment.

RESULTS

Field

Ozone Measurements

Cumulative ozone exposures (ppm*hrs) were similar for both years (113.6 ppm*hrs and 102.5 ppm*hrs in 2000 and 2001, respectively, *see chapter 1*). Exposures at 0.5 m were consistently lower then those at 2 m by approximately 14% (data not shown).

Rainfall data from the nearest weather station at Waterville, NC (12 km away, and 1085 m lower in elevation, 35° 46' N, 83° 06' W) shows that precipitation was 12% lower in 2001 (70.4 cm) compared to 2000 (79.7 cm). There were more dry periods (8) lasting six or more days in 2001 than in 2000 (3). Temperature trends for mean maximum values did not depart from the long term normals by more than 5% in either year (National Climatic Data Center, 2003).

Gas Exchange

Photosynthetic rates of upper and lower leaves were linearly related to PAR at < 75 umol m^{-2} s^{-1} (Fig. 1a & b) in both 2000 and 2001. There were no significant differences ($p>0.05$) in the slopes of these responses between upper and lower leaves (Table 1). On the other hand, stomatal conductance of upper and lower leaves had little or no response to PAR (Fig. 1c & d) in either year. Stomatal conductance was significantly higher ($p=0.0001$) in lower versus upper

leaves in 2000, but that pattern was not evident in 2001 (p=0.437).

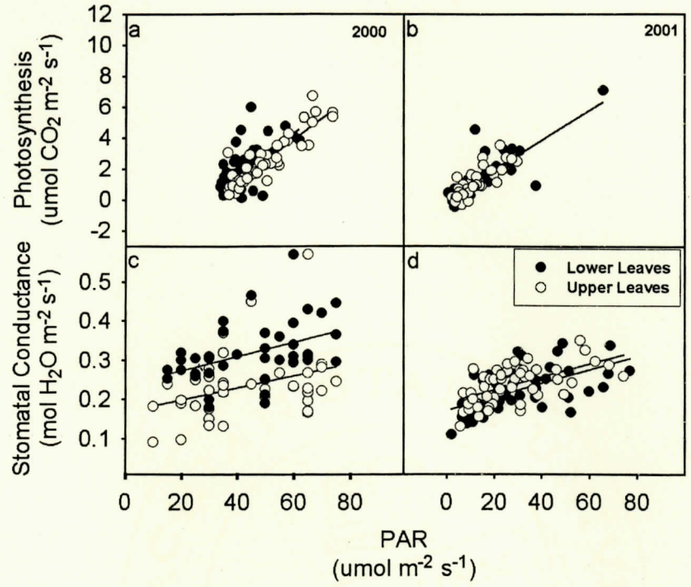

Figure 1. Photosynthesis (a & b) and stomatal conductance (c & d) of upper and lower leaves for 2000 and 2001 at Mt. Sterling Gap. See Table 1 for regression equations.

Light responses for upper and lower leaves were similar in both years, suggesting there were no differences in either carbon uptake, or water use efficiency between leaf positions (Fig. 1). There were too few responses at higher PAR in the field to make any statistical comparisons, but visual inspection of those data did not show any large differences between leaf positions.

Table 1. Regression equations for photosynthetic rates and stomatal conductances of upper and lower leaves.
P_s = Photosynthesis rate in umol CO_2 m^{-2} s^{-1}, G_s = Stomatal conductance in mol H_2O m^{-2} s^{-1}.

	2000			2001		
Leaf Position	Upper	Lower	p	Upper	Lower	p
P_s	y= -0.35 +0.06x r^2= 0.97	y=-0.25 +0.06x r^2= 0.89	0.0001	y=-0.075 +0.04x r^2= 0.59	y=-0.19 +0.04x r^2= 0.69	0.0001
G_s	y=0.23+0.002x r^2= 0.15	y=0.17+0.001x r^2= 0.09	0.0298	y=0.12 +0.002x r^2= 0.34	y=0.09 +0.002x r^2= 0.31	0.0001

Photosynthetic rates in July were similar between sensitive and insensitive individuals in both 2000 and 2001 (Fig. 2a & b). There were no differences in stomatal conductance between sensitive and insensitive individuals in 2001 (p=0.428), and mean conductances in 2000 (which were not separated by sensitivity type) were similar to those in 2001 (Fig. 2c & d).

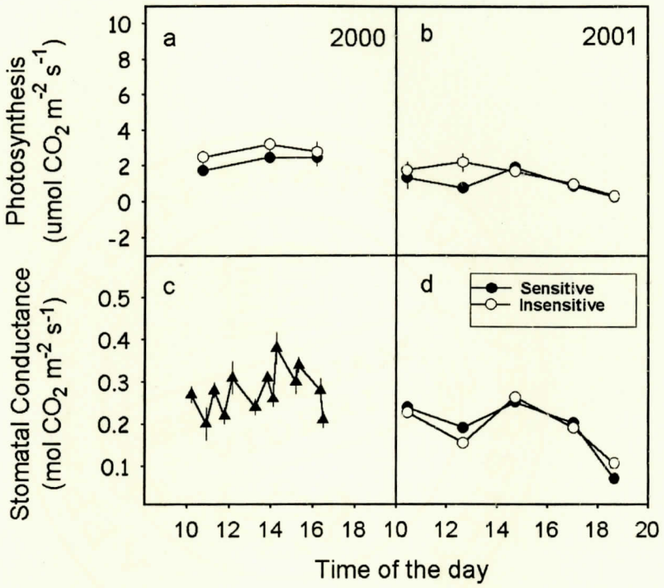

Figure 2. Photosynthesis (a & b) and stomatal conductance (c & d) of sensitive and insensitive individuals vs. time of day at Mt. Sterling Gap for July of 2000 and 2001. Conductivity not measured by sensitivity type in 2000. Values are means ± SE; N = 4-19.

Daily maximum photosynthetic rates in June 2001 did not differ between sensitivity types (p=0.880) but by August, insensitive individuals had higher rates than sensitive ones (p=0.029) (Fig. 3a). There were no significant differences between maximum stomatal conductances for sensitive and insensitive individuals in 2001 (p = 0.730) (Fig 3b), although the trend was for insensitive

individuals to have slightly higher means at both times of the year. Water use efficiencies paralled the patterns for photosynthesis (Fig. 3c).

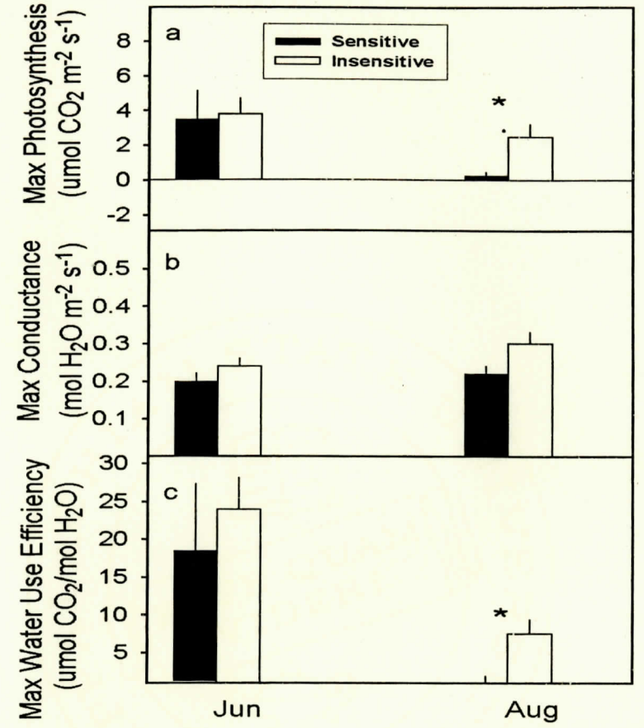

Figure 3. Maximum photosynthesis (a), conductance (b) and water use efficiency (c) of sensitive and insensitive milkweed individuals at Mt. Sterling Gap for June and August , 2001. Asterisks indicate statistical differences between sensitive and insensitive individuals ($p<0.05$). Values are means ± SE; N = 4-11.

Maximum photosynthetic rates and water use efficiencies decreased for both types of plants as the growing season progressed, whereas stomatal conductances remained the same or were slightly higher in August (Fig. 3). Maximum photosynthesis for sensitive individuals was 0.45 ± 0.18 μmol m^{-2} s^{-1} in August, while for insensitive individuals it was 2.1 ± 0.63 μmol m^{-2} s^{-1}. Similarly,

maximum water use efficiency for sensitive individuals was 1.5 ± 0.63 μmol CO_2 /mol H_2O in August, while for insensitive plants it was 6.9 ± 0.63 μmol CO_2 /mol H_2O, respectively.

Biochemistry

Total apoplastic ascorbic acid (oxidized + reduced forms), as well as the redox ratio (amount of reduced relative to oxidized ascorbic acid) were higher earlier in the season (June) of 2001 than later (July and August, p=0.004) (Fig. 4).

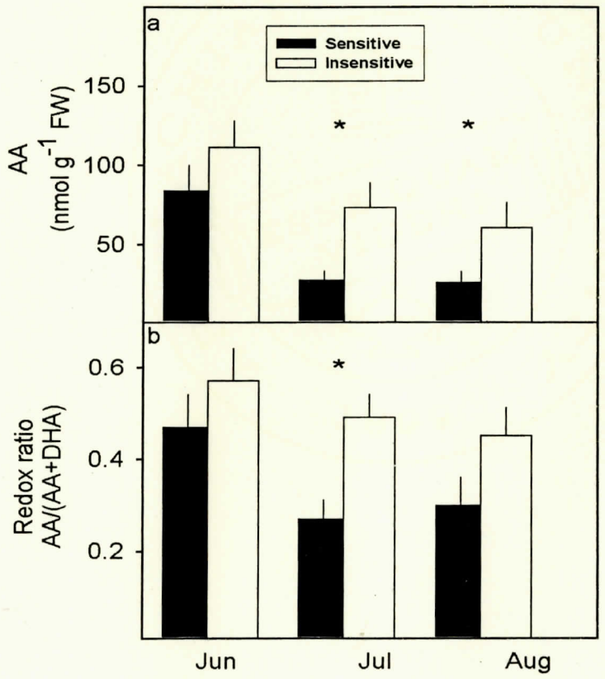

Figure 4. Total apoplastic ascorbate (a) and (b) ratio of reduced to oxidized ascorbic acid for tall milkweeds at Mt.Sterling Gap in June, July and August of 2001. Asterisks indicated statistical differences between sensitive and insensitive leaves ($p<0.05$). Values are means ± SE;N = 6-12. AA=ascorbic acid; DHA=Dehydroascorbic acid.

Amounts of apoplastic ascorbic acid did not differ between sensitivity types in June, but were significantly higher in July and August (p=0.027, Fig. 4a). Mean total apoplastic ascorbic acid amounts for insensitive and sensitive individuals in July were 73.4 ±1.5 nmol g^{-1} fw and 27.9 ± 5.2 nmol g^{-1} fw, respectively, while in August they were 60.3 ±16 nmol g^{-1} fw and 26.4 ± 6.2 nmol g^{-1} fw, respectively. The redox ratio for insensitive plants was higher (0.49 ± 0.05) than that for sensitive plants (0.27 ± 0.04), but only in July (p=0.012) (Fig. 4b).

Greenhouse

Ozone Measurements

Maximum ozone concentrations in the charcoal-filtered chambers never exceeded 19 ppb (Fig. 5), while in the non-filtered chambers they reached a maximum of 59 ppb.

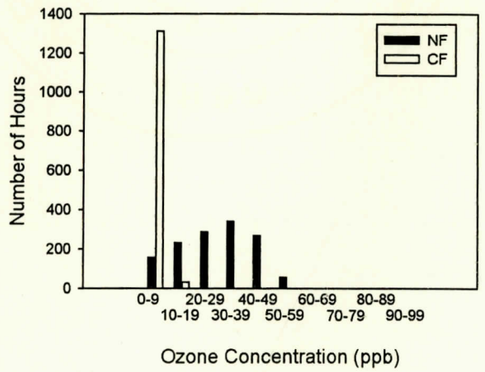

Figure 5. Frequency distributions of ozone concentrations (ppb) in nonfiltered (NF) and charcoal-filtered (CF) chambers.

There were a total of 54 hours above 50 ppb in the NF chambers. The SUM60 (the sum of all ozone concentrations equal to or above 60 ppb) and AOT40 (sum of the differences between 40 ppb and concentrations greater than this value) for the CF chambers were both 0, while in the NF chambers they were 0 and 2.07 ppm*hrs, respectively. The indices were calculated for the period running from June through the middle of October.

Foliar Injury

Ozone-induced foliar injury did not develop in either sensitive or insensitive individuals in either CF or NF chambers.

Gas Exchange

Diurnal Curves

Maximum rates of gas exchange derived from diurnal curves were highest in July (p=0.001), with no significant differences at any other times of the season (Fig. 6a & b). Early in the season (June), insensitive individuals had significantly higher photosynthetic rates (p=0.024) and stomatal conductances (p=0.018) than sensitive individuals, while at no other times during the season were sensitivity types different. Water use efficiencies did not differ among the sensitivity types, but tended to be lower in July and August than June or September (Fig. 6c).

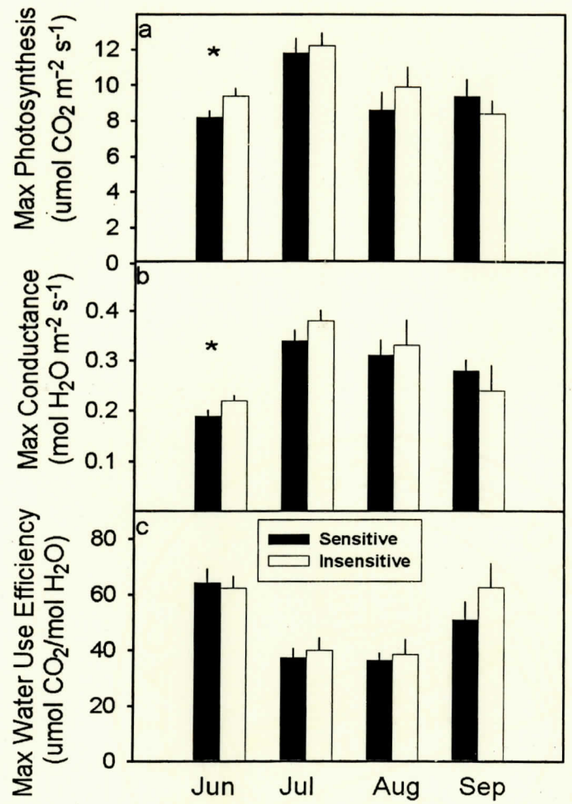

Figure 6. Maximum photosynthesis (a), stomatal conductance (b) and water use efficiency (c) of sensitive and insensitive individuals for June, July, August and September of 2001. Asterisks indicated statistical differences between sensitive and insensitive leaves ($p<0.05$). Values are means ± SE; N = 8.

Light Response Curves

Sensitive and insensitive individuals had similar responses to PAR (Fig. 7a & b). There was a trend for sensitive individuals to have slightly higher photosynthetic rates and stomatal conductances at higher light intensities when compared to insensitive individuals, but these differences were not statistically significant.

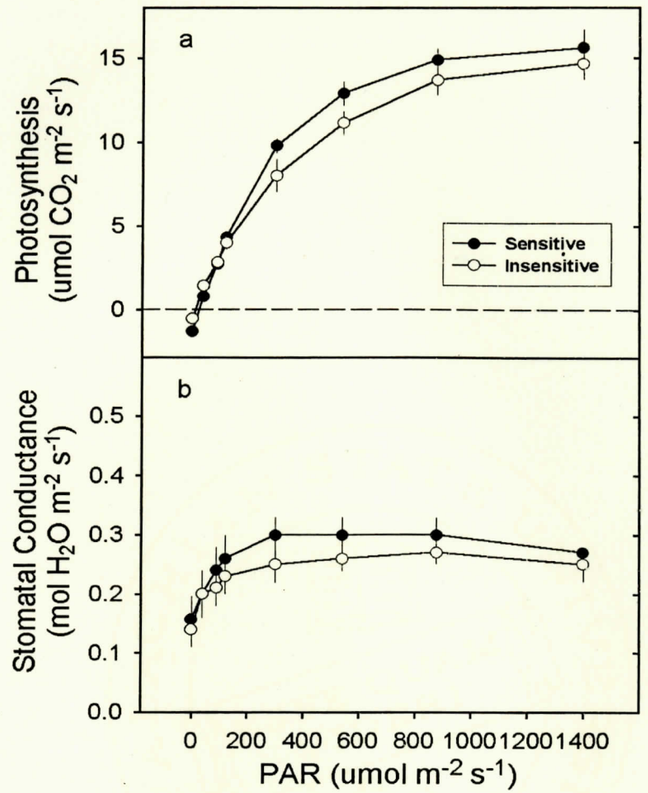

Figure 7. Photosynthesis (a) and stomatal conductance (b) of sensitive and insensitive individuals in filtered and nonfiltered chambers in 2001. Values are means ± SE. N = 8.

Dark respiration and the light compensation point were similar between sensitive and insensitive individuals (Table 2). However, the apparent quantum efficiency was significantly higher for sensitive individuals in both July (p=0.024) and August (p=0.026) (Table 2).

Table 2. Dark respiration, light compensation point and quantum efficiency of sensitive and insensitive individuals in July and August. Values are means (SE). N = 6. Means within a month not followed by a letter, or, followed by same letter are not statistically different (p>0.05).

	July		August	
	Sensitive	**Insensitive**	**Sensitive**	**Insensitive**
Dark Respiration (umol m^{-2} s^{-1})	-1.13 (0.08)	-1.29 (0.34)	-1.25 (0.13)	-1.45 (0.27)
Compensation Point (umol m^{-2} s^{-1})	21.9 (3.1)	29.5 (7.6)	26.5 (2.9)	33.3 (8.5)
Quantum efficiency (umol $umol^{-1}$)	0.040 [a] (0.002)	0.034 [b] (0.002)	0.050 [a] (0.002)	0.040 [b] (0.002)

Belowground Biomass

Initial rhizome weight did not contribute to the variation in belowground biomass. There were no differences in either rhizome or root fresh weights between the insensitive and sensitive genotypes (p=0.819), nor between CF and NF grown plants (p=0.098) (Table 3).

Table 3. Root fresh weight of sensitive and insensitive individuals in charcoal-filtered (CF) and non-filtered (NF) chambers. Values are means (SE). N = 4.

	Sensitive		**Insensitive**	
Treatment	**CF**	**NF**	**CF**	**NF**
Roots	**26.5 (7)**	**36.6 (2)**	**26.4 (5)**	**39.1 (5)**

DISCUSSION

The existence of genetic variation in sensitivity of some plant species to ozone has been known for sometime (Berrang et al., 1989 & 1991; Reiling and Davison, 1992a; Taylor, 1994; Karnosky et al., 1996; Davison & Barnes, 1998; Lee et al., 1999). Only recently, however, have concerted efforts been made to determine those factors responsible for these differences. Initially, investigators concentrated on differences in uptake via stomata as a factor determining susceptibility to ozone, with sensitivity expressed in terms of the internal dosage rather than the external exposure (Evans & Ting, 1974;Taylor, 1978; Taylor et al., 1982; Reich, 1987; Runeckles, 1992; Bytnerowicz, 1996; Bungener et al., 1999).

A variety of patterns have been found in the literature in terms of the response of stomata to ozone, which include closing (Nali et al., 1998; Ranieri et al., 1999; Sober & Sild, 1999; Guidi et al., 2001), no effects (Clark et al., 1996), and even opening (Mansfield & Pearson, 1996). In addition, ozone may cause stomatal sluggishness, which slows both the opening and closing responses relative to changes in the micro-climate surrounding the stomata (Maier-Maercker, 1998; McAinsh et al., 2002). Pasqualini et al. (2002) showed, for example, that ozone caused stomatal closure in both sensitive and insensitive varieties of tobacco, but stomatal recovery was greater in the insensitive variety after removal from ozone exposure. Thus, stomatal sensitivity to ozone may be

related not only to the duration of exposure, but also to its ability to recover during periods of low ozone.

In my study, I found few differences in gas exchange in field plants between sensitive and insensitive genotypes of tall milkweed with respect to either photosynthesis or stomatal conductance (Fig.2). The exception was late in the season, when senescence was more pronounced in the sensitive individuals than insensitive ones *(see chapter 1)*. As a consequence, photosynthesis was greatly reduced in sensitive plants at this time (Fig.3). Since most of the foliar injury had already occurred by this date, I do not feel that this was a cause of greater sensitivity in this group of plants so much as a consequence of greater sensitivity. Despite the large reduction in photosynthesis later in the growing season, stomatal conductance was not decreased. Thus, for most of the season, there were few if any differences in stomatal conductance between sensitivity types (Fig.3). In addition, there were few differences in the ratio of photosynthesis to conductance (what I termed water use efficiency) until the last sampling date. Therefore, I conclude that sensitivity is not related to the ratio of carbon fixed to ozone absorbed, as Fredericksen et al. (1995) have speculated might be the case in black cherry (*Prunus serotina*) trees.

Under controlled conditions (in open-top chambers) outside the greenhouse, I also did not find any evidence for a difference in stomatal conductance between sensitive and insensitive individuals, either using diurnal curves done outside near the OTCs (Fig. 6), or in the lab during my light response curves (Fig. 7). In addition, there were no differences between

sensitivity types in either CF or NF air. Therefore, considering both the field and greenhouse experiments, I find little support for the hypothesis that sensitivity is related to stomatal conductance (i.e. uptake of ozone). This agrees with Zhang et al. (2001), who also failed to find a relationship between sensitivity of native plant species in Switzerland and stomatal conductance. If differences in uptake are not the reason for variation in sensitivity to ozone, then other factors must be considered as potential mechanisms (Runeckles, 1992). The two most likely causes of differential sensitivity include leaf structural differences that influence the distribution of ozone to the mesophyll cells (Evans & Ting, 1974; Evans et al., 1996), and differences in the anti-oxidant capabilities of sensitive and insensitive plants (Lyons et al.,1999; Burkey et al., 2000; Zheng et al., 2000).

The only gas exchange parameter in the light response curves that differed between sensitive and insensitive plants was the apparent quantum efficiency, which was higher in sensitive plants by about 20-25% (Table 2). Since quantum efficiency is a measure of the light response for electron transport when light is limiting, a higher efficiency might be indicative of a thinner leaf. This would reduce self-shading within the leaf, thereby raising the quantum efficiency. Bennett et al. (1992) have shown that sensitivity to ozone is greater in plants with thinner compared to thicker leaves. However, Ferdinand et al. (2000) found just the opposite. In actuality, sensitivity might be best correlated with the amount of internal cell wallspaces exposed to air (Evans et al., 1996), which would facilitate diffusion of ozone to these mesophyll and palisade cells. Since leaf thickness is not necessarily correlated with the amount of internal cell wallspaces (James et

al., 1999), this could explain the discrepancy between Bennett et al. (1992) and Ferdinand et al. (2000). There is even some evidence that exposure to ozone can cause increases in leaf thickness (Lawson et al., 2002).

I could not detect a similar difference in quantum efficiency between these same genotypes when measuring photosynthesis in the field. Perhaps leaves in the field were not at steady-state conditions, and environmental conditions were more variable, thereby obscuring the small differences in quantum efficiency found in the greenhouse. Nonetheless, the potential difference in quantum efficiency under controlled conditions does suggest that leaf anatomical differences may play a role in determining the sensitivity of these plants to ozone. Further studies of the influence of shading and ozone on leaf internal anatomical characteristics are needed, as well as studies on potential impacts of leaf phenology on leaf anatomy (*see chapter 1*). Such measurements, when combined with stomatal conductance data and anti-oxidant capabilities, may prove useful in parameterizing models of ozone sensitivity in plants (Plochl et al., 2000).

There was a tendency for the apoplastic ascorbic acid content to be higher later in the season for insensitive individuals compared to sensitive ones (Fig. 4). In addition, the redox ratio was higher in July than at other times. Together, these data suggest that perhaps sensitivity may result partially from a difference in the anti-oxidant capabilities of the two sensitivity types. Insensitive milkweeds have higher ascorbic acid contents in the extracellular area, as well as a higher percentage of reduced to oxidized ascorbic acid (redox ratio), and thus have

percentage of reduced to oxidized ascorbic acid (redox ratio), and thus have more AA available to detoxify incoming ozone. Other studies, using mainly crop plants, have shown some correlation of apoplastic ascorbic acid content and sensitivity (Luwe et al., 1993 ; Burkey, 1999; Lyons et al., 1999; Zheng et al., 2000; Robinson & Britz, 2001), while others were not able to demonstrate this same correlation (Polle et al., 1999; Yun & Laurence, 1999; Burkey et al., 2000). Most studies though, do agree that even if sensitivity is affected by ascorbic acid content, there is not enough present in the apoplastic spaces to totally protect a leaf against ozone injury (Chameides, 1989; Luwe & Heber, 1995; Ranieri et al., 1999; Burkey & Eason, 2002; Kollist et al., 2000). Therefore, resistance to ozone in certain genotypes can not be due solely to variation in ascorbic acid amounts.

The seasonal decline in ascorbic acid content in the tall milkweeds in my study roughly correlates with the timing of first injury symptoms observed in the field *(see Chapter 1 for a discussion of phenological trends in foliar injury).* Although, this does not prove that the decrease in ascorbic acid content is the reason why foliar injury shows up at this time, it does suggest that the two might be related. Only further studies, under more controlled conditions, can unequivocally answer this question.

Finally, plants can produce anti-oxidants other than ascorbic acid, such as glutathione and alpha-tocopherol (Rao et al., 1996; Wieser et al., 2001), and sensitivity may be related more to the total anti-oxidant capability of a leaf rather than to ascorbic acid alone. Studies are currently underway to evaluate the total anti-oxidant status of my tall milkweed plants.

Conclusions

My data strongly suggest that differences between sensitive and insensitive individuals are not the result of differential ozone uptake arising from differences in stomatal conductances. I do, however, have preliminary evidence that leaf anatomy and biochemical anti-oxidant status may be contributing factors.

Further studies should concentrate on elucidating the potential influences of these latter two factors on ozone sensitivity, while additional gas exchange measurements could be made to determine if perhaps ozone causes transient responses in one sensitivity type and not the other (Pasqualini et al., 2002), and whether ambient levels of ozone are high enough to cause detectable changes in conductance, irrespective of sensitivity type. A combination of both field and controlled chamber studies will be needed to answer these questions.

Berrang P, Karnosky DF, Bennett JP. 1991. Natural selection for ozone tolerance in *Populus tremuloides*: an evaluation of nationwide trends. *Canadian Journal Forest Research* **21:**1091-1097.

Bergweiler CJ, Manning WJ. 1999. Inhibition of flowering and reproductive success in spreading dogbane *(Apocynum androsaemifolium*) by exposure to ambient ozone. *Environmental Pollution* **105:** 333-339.

Blum O, Bytnerowicz A, Manning W, Popovicheva L. 1997. Ambient tropospheric ozone in the Ukranian Carpathian mountains and Kiev region: detection with passive samplers and bioindicator plants. *Environmental Pollution* **98:** 299-304.

Braun S, Fluckiger W. 1995. Effects of ambient ozone on seedlings of *Fagus sylvatica* L. and *Picea abies* (L.) Karst. *New Phytologist* **129:**33-44.

Bungener P, Balls GR, Nussbaum S, Geissmann M, Grub A, Fuhrer J. 1999. Leaf injury characteristics of grassland species exposed to ozone in relation to soil moisture condition and vapour pressure deficit. *New Phytologist* **142:** 271-282.

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

Burkey KO, Eason G. 2002. Ozone tolerance in snap bean is associated with elevated ascorbic acid in the leaf apoplast. *Physiologia Plantarum* **114:** 387-394.

Burkey KO, Neufeld HS, Souza L, Chappelka AH, Davison AW. 2001. Seasonal Profiles of Leaf Ascorbic Acid in Ozone-Sensitive Wildflowers. Poster presented at 34th Air Pollution Workshop, Penn State University, State College, PA.

Burkey KO, Wei C, Eason G, Ghosh P, Fenner GP. 2000. Antioxidant metabolite levels in ozone-sensitive and tolerant genotypes of snap bean. *Physiologia Plantarum* **110** :1-6.

Bytnerowicz A. 1996. Physiological aspects of air pollution stress in forests. *Phyton-Horn.* **36:**15-22.

Bytnerowicz A, Manning WJ, Grosjean D, Chielewski W, Dmuchowski W, Grodzinska K, and Godzik B. 1993. Detecting ozone and demonstrating its phytotoxicity in forested areas in Poland: a pilot study. *Environmental Pollution* **80:**301-305.

Calatayud A, Ramirez JW, Iglesias DJ, Barreno E. 2002. Effects of ozone on photosynthesis CO_2 exchange, chlorophyll a fluorescence and antioxidant systems in lettuce leaves. *Physiologia Plantarum* **116:** 308-316.

Chameides WL. 1989. The chemistry of ozone deposition to plant leaves: the role of ascorbic acid. *Environment, Science, Technology* **23:** 595-600.

Chameides WJ, Cowling EB. 1995. The state of the southern oxidant study (SOS): Policy-relevant findings in ozone pollution research 1988-1994. SOS. Coll. For.Res., NC St. Univ., Raleigh, NC, 94pp.

Chappelka AH, Neufeld HS, Davison AW, Somers GL, Renfro JR. 2003. Ozone injury on cutleaf coneflower (*Rudbekia laciniata*) and crown-beard (*Verbesina occidentalis*) in Great Smoky Mountains National Park. *Environmental Pollution (in press).*

Chappelka AH, Renfro JR, Somers G, Nash B. 1997. Evaluation of ozone injury on foliage of black cherry (*Prunus serotina*) and tall milkweed (*Asclepias exaltata*) in Great Smoky Mountains National Park . *Environmental Pollution* **95:**13-18.

Chappelka AH, Samuelson LJ. 1998. Ambient ozone effects on forest trees of the eastern United States: a review. *New Phytologist* **139:** 91-108.

Chappelka AH, Wergowske DJ. 1993. Foliar ozone injury surveys in national forests in Alabama and Mississippi. In South. For. Dis. Workshop, 13-15 Jan., Auburn, AL, pp. 27.

Castillo FJ, Greppin H. 1988. Extracellular ascorbic acid and enzyme activities related to ascorbic acid metabolism in *Sedum album* L. leaves after ozone treatment. *Environmental & Experimental Botany* **28**: 231-238.

Clark CS, Weber JA, Lee EH, Hogsett WE. 1996. Reductions in gas exchange of *Populus tremuloides* caused by leaf aging and ozone exposure. *Canadian Journal of Forest Research* **26:**1384-1391.

Conklin PL, Williams EH, Last RL. 1996. Environmental stress sensitivity of an ascorbic acid-deficient *Arabidopsis* mutant. Proc Natl Acad Sci USA **93**: 9970-9974.

Dalstein L, Torti X, Le-Thiec D, Dizengremel P. 2002. Physiological study of declining *Pinus sembra* (L.) trees in southern France. *Trees Berlin* **16:** 299-305.

Dattore RE, Gebhart KA, Malm WC, Flores M. 1991. Use of an atmospheric trajectory model to explore the source regions affecting ozone concentrations at five eastern U.S. national parks. Air and Waste Management Association. 84th Annual Meeting & Exhibition. Vancouver, British Columbia. 91:1-

Davis DD, Skelly JM. 1992. Foliar sensitivity of eight eastern hardwood tree species to ozone. *Water, Air Soil Pollution* **62:**269-277.

Davison AW, Barnes JD. 1998. Effects of ozone on wild plants. *New Phytologist* **139**:135-151.

Department of the Interior. 1982. Preliminary certification of no adverse impact on Theodore Roosevelt Park and Lostwood National Wildlife Refuge under Section 165(d)(2)(C)iii of the Clean Air Act, Federal Registry 47 (133), 3022-3024.

Dietz KJ. 1997. Functions and responses of the leaf apoplast under stress. *Prog Botany* **58:** 221-254.

Drogoudi PD, Ashmore MR. 2000. Does elevated ozone have differing effects in flowering and deblossomed strawberry? *New Phytologist* **147:**561-569.

Elagoz V, Manning WJ. 2002. Ozone and bean plants: morphology matters. *Environmental Pollution* **120:** 521-524.

Evans LS, Adamski JH, Renfro JR. 1996. Relationships between anatomical characteristics and ozone sensitivity of leaves of several herbaceous dicotyledonous plant species at Great Smoky Mountains National Park. *Environmental & Experimental Botany* **36:** 413-420.

Evans LS, Ting JP. 1974. Ozone sensitivity of leaves; relationship to leaf water content, gas transfer resistance and anatomical characteristics. *American Journal of Botany* **61:** 592-597.

Ferdinand JA, Fredericksen TS, Kouterick KB, Skelly JM. 2000. Leaf morphology and ozone sensitivity of two open pollinated genotypes of black cherry (*Prunus serotina*) seedlings. *Environmental Pollution* **108:** 297-302.

Fredericksen TS, Joyce BJ, Skelly JM, Steiner KC, Kolb TE, Kouterick KB, Savage JE. 1995. Physiology, morphology, and ozone uptake of leaves of black cherry seedlings, saplings, and canopy trees. *Environmental Pollution* **89:** 273-283.

Fryer MJ. 1992. The antioxidant effects of thylakoid membrane vitamin E. *Plant, Cell & Environment* **15**: 381-392.

Fujinuma Y, Furukawa A, Aiga I. 1988. Effect of ozone on stomatal conductance in sunflower leaves age-dependent inhibition. *Environment Control in Biology* **26:**83-90.

Ghosh S, Skelly JM, Innes JL, Skelly L. 1998. Temporal development of visual ozone injury on the foliage of *Prunus serotina*- a statistical evaluation. *Environmental Pollution* **102:** 287-300.

Gravano E, Giulietty V, Desotgiu R, Bussotti F, Grossoni P, Gerosa G, Tani C. 2003. Foliar response of an *Ailanthus altissima* clone in two sites with different levels of ozone-pollution. *Environmental Pollution* **121:** 137-146.

Guidi L, Nali CL, Lorenzini G, Fillippi F, Soldatini GF. 2001. Effects of chronic ozone fumigation on the photosynthetic process of poplar clones showing different sensitivity. *Environmental Pollution* **113**: 245-254.

Gunthardt-Goerg MS, Matyssek R, Scheidegger C, Keller T. 1993. Differentiation and structural decline in the leaves and bark of birch (*Betula pendula*) under low ozone concentrations. *Trees* **7:** 104-114.

Gunthardt-Goerg MS, McQuattie CJ, Maurer S, Frey B. 2000. Visible microscopic injury in leaves of five deciduous tree species related to current critical ozone levels. *Environmental Pollution* **109:** 489-500.

Gruenhage L, Jaeger HJ, Haenel DH, Loepmeier FJ, Hanewald K. 1999. The European critical levels for ozone: improving their usage. *Environmental Pollution* **105:** 163-173.

Heagle AS, Miller JE, Chevone BI, Dreschel TW, Manning WJ, McPool PM, Morrison CL, Neely GE, and Rebbeck J. 1995. Response of white clover indicator system to tropospheric ozone at eight locations in the United States. *Water, Air and Soil Pollution* **85:** 1373-1378.

Heagle AS, Miller JE, Sherrill DE. 1994. A white clover system to estimate effects of tropospheric ozone on plants. *Journal of Environmental Quality* **23:** 613-621.

Hildebrand E, Skelly JM, Fredericksen TS. 1996. Foliar response of ozone-sensitive hardwood tree species from 1991 to 1993 in the Shenandoah National Park, Virginia. *Canadian Journal of Forest Research* **26:** 658-669.

Holley JD, Hofstra G, Hall R. 1985. Appearance and fine structure of lesions caused by the interactions of ozone and *Alternaria solani* in potato leaves. *Canadian Journal of Plant Pathology* **7:** 277-282.

Horsfall JG, Barratt RW. 1945. An improved grading system for measuring plant disease. *Phytopathology* **35:**655.

James SA, Smith WK, Vogelmann TC. 1999. Ontogenetic differences in Mesophyll structure and chlorophyll distribution in *Eucalyptus globulus* spp. *globulus* (Myrtaceae). *Americal Journal of Botany* **86:** 198-207.

Kang D, Aneja VP, Zika RG, Farmer C, Ray JD. 2001. Nonmethane hydrocarbons in the rural southeast United States national parks. *Journal of Geophysical Research* **106:** 3133-3155.

Karlsson GP, Sellden G, Skarbyk L, Pleijel H. 1995. Clover as an indicator plant for phytotoxic ozone conentrations: Visible injury in relation to species, leaf age, and exposure dynamics. *New Phytologist* **129:** 355-365.

Karnosky DF, Gagnon ZE, Dickson RE, Coleman MD, Lee EH, Isebrands JG. 1996. Changes in growth, leaf abcission, and biomass associated with seasonal tropospheric ozone exposure of *Populus tremuloides* clones and seedlings. *Canadian Journal of Forest Research* **26:**23-37.

Keller T. 1988. Growth and premature leaf fall in American aspen as bioindicators for ozone. *Environmental Pollution* **52:** 183-192.

Kelly FJ, Mudway I, Krishna MT, Holgate ST. 1995. The free radical basis of air pollution: focus on ozone. *Respir Med* **89**: 647-656.

Kolb TE, Matyssek R. 2001. Limitations and perspectives about scaling ozone impacts on trees. *Environmental Pollution.* **115:**373-393.

Kollist H, Moldau H, Mortensen L, Rasmussen SK, Jorgensen L. 2000. Ozone flux to plasmalemma in barley and wheat is controlled by stomata rather than by direct reaction of ozone with cell wall ascorbate. *Plant Physiology* **156:** 645 651.

Krupa SV. 2000. Ultraviolet-B radiation, ozone and plant biology. *Environmental Pollution* **110:** 193-194.

Krupa SV, Manning WJ. 1988. Atmospheric ozone: formation and effects on vegetation. *Environmental Pollution* **50:**101-137.

Krupa SV, Nosal M, Peterson DL. 2001. Use of passive ambient ozone samplers. *Environmental Pollution* **112:** 303-309.

Lawson T, Craigon J, Black CR, Colls JJ, Landon G, Weyers-Jonathan DB. 2002. Impact of elevated CO_2 and O_3 on gas exchange parameters and epidermal characteristics in potato (*Solanum tuberosum* L.). *Journal of Experimental Botany* **53:** 737-746.

Lee JC, Skelly JM, Steiner KC, Zhang JW, Savage JE. 1999. Foliar response of black cherry (*Prunus serotina*) clones to ambient ozone exposure in central Pennsylvania. *Environmental Pollution* **105:**325-331.

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

Luwe MWF, Takahama U, Heber U. 1993. Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia oleraceae* L.) leaves. *Plant Physiology* **101:** 969-976.

Lyons T, Plochl M, Turcsanyi E, Barnes J. 1999. Extracellular antioxidants: a protective screen against ozone? In: Agrawal S, Agrawal M (eds) Environmental Pollution and Plant responses. CRC Press Lewis Publishers. Boca Raton, pp. 183-201.

Maier-Maercker U. 1998. Predisposition of trees to drought stress by ozone. *Tree Physiology* **19:** 71-78.

Manning WJ. 1993. Bioindicator plants for assessment of air quality: General considerations and plant responses to ambient ozone. In Proc: 86th Ann. Meet. Air and Waste Manage. Assoc., 13-18 June 1993, Denver, CO, preprint 93-WA80.01., pp. 14.

Manning WJ, Godzik B, Musselman R. 2002. Potential bioindicator species for ambient ozone in forested mountain areas of central Europe. *Environmental Pollution* **119:** 283-290.

Mansfield TA, Pearson M. 1996. Disturbances in stomatal behaviour in plants exposed to air pollution. In: Yunus, M, Iqbal, M (eds). Plant Response to Air Pollution. Chichester, pp, 135-166.

McAinsh MR, Evans NH, Montogomery LT, North KA. 2002. Calcium signaling in stomatal responses to pollutants. *New Phytologist* **153:** 441-447.

McKee IF, Farage PK, Long SP. 1995. The interactive effects of elevated CO_2 and ozone concentration on photosynthesis in spring wheat. *Photosynthesis Research* **45:** 111-119.

McLaughlin SB, Downing DJ. 1985. Effects of air pollutants on forests: a critical review. *Journal of Air Pollution Control Association* **35:** 516-534.

McLaughlin SB, McConathy RK, Duvick D, Mann LK. 1982. Effects of chronic air pollution stress on photosynthesis carbon allocation and growth of white pine trees *Pinus strobus*. *Forest Science* **28:** 60-70.

Mueller SF. 1994. Characterization of ambient ozone levels in Great Smoky Mountains National Park. *Journal of Applied Meteorology* **33**:465-72.

Muller R. 2003. Nutrient relations of the herbaceous layer in deciduous forest ecosystems. In: Gilliam, FS, Roberts, MR (eds), The Herbaceous Layer in Forests of Eastern North America, New York, NY, (*in press*).

Nali C, Guidi L, Filippi F, Soldatini GF, Lorenzini G. 1998. Photosynthesis of two poplar clones contrasting in ozone sensitivity. *Trees Berlin* **12:** 196-200.

National Climatic Data Center. 2003. http:// www.ncdc.noaa.gov

National Research Council. 1991. Rethinking the ozone problem in urban and regional air pollution. National Academy Press, Washington DC, 500 pp.

Nebel B, Fuhrer J. 1994. Inter-and intraspecific differences in ozone sensitivity in semi-natural plant communities. *Angewandte-Botanik* **68:** 116-121.

Neufeld HS, Renfro JR, Hacker WD, Silsbee D. 1992. Ozone in Great Smoky Mountains National Park: dynamics and effects on plants. In: Berglund, R.D. (Ed.), Tropospheric Ozone and the Environment II. Air and Waste Management Association, Pittsburgh, PA, pp. 594-617.

Neufeld HS, Young DR. 2003. Ecophysiology of the herbaceous layer in temperate and deciduous forests. In: Gilliam, FS & Roberts, MR (Eds), The Herbaceous Layer in Forests of Eastern North America, New York, NY, (*in press*).

Olszyk DM, Tibbitts TW. 1981. Stomatal responses and leaf injury in *Pisum sativum* cultivar *alsweet* with sulfur dioxide and ozone exposures 1. Influence of pollutant level and leaf maturity. *Plant Physiology* **67:**539-544.

70

McKee IF, Farage PK, Long SP. 1995. The interactive effects of elevated CO_2 and ozone concentration on photosynthesis in spring wheat. *Photosynthesis Research* **45:** 111-119.

McLaughlin SB, Downing DJ. 1985. Effects of air pollutants on forests: a critical review. *Journal of Air Pollution Control Association* **35:** 516-534.

McLaughlin SB, McConathy RK, Duvick D, Mann LK. 1982. Effects of chronic air pollution stress on photosynthesis carbon allocation and growth of white pine trees *Pinus strobus*. *Forest Science* **28:** 60-70.

Mueller SF. 1994. Characterization of ambient ozone levels in Great Smoky Mountains National Park. *Journal of Applied Meteorology* **33**:465-72.

Muller R. 2003. Nutrient relations of the herbaceous layer in deciduous forest ecosystems. In: Gilliam, FS, Roberts, MR (eds), The Herbaceous Layer in Forests of Eastern North America, New York, NY, (*in press*).

Nali C, Guidi L, Filippi F, Soldatini GF, Lorenzini G. 1998. Photosynthesis of two poplar clones contrasting in ozone sensitivity. *Trees Berlin* **12:** 196-200.

National Climatic Data Center. 2003. http:// www.ncdc.noaa.gov

National Research Council. 1991. Rethinking the ozone problem in urban and regional air pollution. National Academy Press, Washington DC, 500 pp.

Nebel B, Fuhrer J. 1994. Inter-and intraspecific differences in ozone sensitivity in semi-natural plant communities. *Angewandte-Botanik* **68:** 116-121.

Neufeld HS, Renfro JR, Hacker WD, Silsbee D. 1992. Ozone in Great Smoky Mountains National Park: dynamics and effects on plants. In: Berglund, R.D. (Ed.), Tropospheric Ozone and the Environment II. Air and Waste Management Association, Pittsburgh, PA, pp. 594-617.

Neufeld HS, Young DR. 2003. Ecophysiology of the herbaceous layer in temperate and deciduous forests. In: Gilliam, FS & Roberts, MR (Eds), The Herbaceous Layer in Forests of Eastern North America, New York, NY, (*in press*).

Olszyk DM, Tibbitts TW. 1981. Stomatal responses and leaf injury in *Pisum sativum* cultivar *alsweet* with sulfur dioxide and ozone exposures 1. Influence of pollutant level and leaf maturity. *Plant Physiology* **67:**539-544.

Paakkonen E, Holopainen T, Karenlampi L. 1997. Variation in ozone sensitivity among clones of *Betula pendula* and *Betulal pubescens*. *Environmental Pollution* **95**:37-44.

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

Patterson MC, Samuelson L, Somers G, Mays A. 2000. Environmental control of stomatal conductance in forest trees of the Great Smoky Mountains National Park. *Environmental Pollution* **110:**225-233.

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

Plochl M, Lyons T, Ollerenshaw J, Barnes J. 2000. Simulating ozone detoxification in the leaf apoplast through direct reaction with ascorbate. *Planta* **210:** 454-467.

Polle A, Rennenberg H, Scholz F. 1999. Antioxidative systems in spruce clones grown under high altitudes. *Phyton-Horn.* **39:** 155-164.

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

Rao MV, Gopinadhan P, Douglas PO. 1996. Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana. Plant Physiology* **110:** 125-136.

Reich PB. 1987. Quantifying plant responses to ozone: a unifying theory. *Tree Physiology* **3:** 63-91.

Reiling K, Davison AW. 1992a. The response of native, herbaceous species to ozone: growth and fluorescence screening. *New Phytologist* **121:**643-647.

Reiling K, Davison AW. 1992b. Effects of a short ozone exposure given at different stages of development of *Plantago major* L. *New Phytologist* **121:** 29-37.

Ribas A, Filella I, Gimeno BS, Penuelas J. 1998. Evaluation of tobacco cultivars as bioindicators and biomonitors of ozone phytotoxic levels in Catalonia. *Water, Air and Soil Pollution* **107:** 347-365.

Robinson JM, Britz SJ. 2001. Ascorbate-dehydroascorbate level and redox status in leaflets of field-grown soybeans exposed to elevated ozone. *International Journal of Plant Sciences* **162:** 119-125.

Runeckles VC. 1992. Uptake of ozone by vegetation. In: Allen S. Lefohn (ed) Surface level ozone exposures and their effects on vegetation. Lewis Publishers, Inc. Chelsea, pp. 157-188.

Scebba F, Soldatini G, Ranieri A. 2003. Ozone differentially affects physiological and biochemical responses of two clover species; *Trifolium repens* and *Trifolium pretense. Environmental Pollution* **123:** 209-216.

Schaub M, Skelly JM, Steiner KC, Davis DD, Pennypacker SP, Zhang J, Ferdinand JA, Savage JE, Stevenson RE. 2003. Physiological and foliar injury responses of *Prunus serotina*, *Fraxinus americana*, and *Acer rubrum* seedlings to varying soil moisture and ozone. *Environmental Pollution (in press).*

Shannon TR, Wyatt R. 1986. Reproductive biology of *Asclepias exaltata. American Journal of Botany.* **73:** 11-20.

Shaver CL, Tonnessen KA, Maniero TG. 1994. Clearing the air at the Great Smoky Mountains National Park. *Ecological Applications* **4:** 690-701.

Skelly JM, Innes JL, Savage JE, Snyder KR, Vanderheyden D, Zhang J, Sanz MJ. 1999. Observation and confirmation of foliar symptoms of native plant species of Switzerland and Southern Spain. *Water, Air and Soil Pollution* **116:** 227-234.

Skelly JM, Savage JE, De-Bauer ML, Alvarado D. 1997. Observations of ozone-induced foliar injury on black cherry (*Prunus serotina*, var. *capuli*) within the Desierto de Los Leones National Park, Mexico City. *Environmental Pollution* **95:** 155-158.

Sober A, Sild E. 1999. Stomatal closure during tobacco leaf desiccation as affected by ozone. *Biologia Plantarum* **42:** 267-272.

Staedtler S, Ziegler H. 1993. Illustration of the genetic differences in ozone sensitivity between the varieties *Nicotiana tabacum* Bel W-3 and Bell B using various plant systems. *Botanica-Acta* **106:** 265-276.

Tanaka K, Suda Y, Kondo N, Sugahara K. 1985. Ozone tolerance and the ascorbate-dependent hydrogen peroxide decomposing system in chloroplasts. *Plant, Cell & Physiology* **26**: 1425-1431.

Tegischer K, Tauz M, Weiser G, Grill D. 2002. Tree-and needle-age-dependent variations in antioxidants and photoprotective pigments in Norway spruce needles at the alpine timberline. *Tree Physiology* **22:**591-596.

Taylor GE. 1978. Plant and leaf resistance to gaseous air pollution stress. *New Phytologist* **80:** 523-534.

Taylor GE. 1994. Role of genotype in the response of loblolly pine to tropospheric ozone: Effects at the whole-tree, stand, and regional level. *Journal of Environmental Quality* **23:** 63-82.

Taylor GE, Jr., Tingey DT, Ratsch HC. 1982. Ozone flux in *Glycine max.* (L.) Merr.: sites of regulation and relationship to leaf injury. *Oecologia* **53:** 179-186.

Taylor MD, Sinn JP, Davis DD, Pell EJ. 2002. The impact of ozone on a salt marsh grass (*Spartina alterniflora*). *Environmental Pollution* **120:**701-705.

Torsethaugen G, Pell EJ, Assmann SM. 1999. Ozone inhibits guard cell K+ channels implicated in stomatal opening. Proceedings of the National Academy of Sciences of the United States of America. **96:** 13577-13582.

US EPA. 1996. Air Quality Criteria for Ozone and Other Photochemical Oxidants. Vol. II EPA-600/P-93/00bF. US EPA, National Center for Environmental Assessment. Research/Triangle Park, NC.

US EPA. 2001. Latest finds on national air quality: 2000 status and trends. EPA/454/K-01-002. Office of Air Quality, Planning and Standards, US Environmental Protection Agency, Research Triangle Park, NC.

USDA. 2003. http://plants.usda.gov/plants/

VanderHeyden D, Skelly J, Innes J, Hug C, Zhang J, Landolt W, Bleuler 2001. Ozone exposure thresholds and foliar injury on forest plants in Switzerland. *Environmental Pollution* **111:**321-331.

Wieser G, Tausz M, Wonisch A, Havranek WM. 2001. Free radical scavengers and photosythetic pigments in *Pinus cembra* L. needles affected by ozone exposure. *Biologia Plantarum-Prague* **44:** 225-232.

Wiltshire JJJ, Wright CJ, Colls JJ, Unsworth, Craigon, J. 1993. The effect of ozone episodes on autumn leaf fall in apple. *New Phytologist* **124:**433-437.

Whittaker RH. 1956. The vegetation of the Great Smoky Mountains. *Ecological Monographs* **26:** 1-80.

Yun SC, Laurence JA. 1999. The response of clones *Populus tremuloides* differing in sensitivity to ozone in the field. *New Phytologist.* **141:** 411-421.

Zar JH. 1999. Biostatistical Analysis, 4th edition, Prentice Hall, Upper Saddle River, NJ. pp. 122-159.

Zhang J, Ferdinand JA, Vanderheyden DJ, Skelly JM, Innes JL. 2001. Variation in gas exchange within native plant species of Switzerland and relationships with ozone injury: An open-top experiment. *Environmental Pollution* **113:** 177-185.

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

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