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By: **H.S. Neufeld**, E.H. Lee, J.R. Renfro, and W.D. Hacker

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Seedling insensitivity to ozone for three conifer species native to Great Smoky Mountains National Park☆

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"Capsule": *Ambient concentrations of ozone had little effect on seedlings of three species of conifers commonly found in Great Smoky Mountains National Park.*

Abstract

Field symptoms typical of ozone injury have been observed on several conifer species in Great Smoky Mountains National Park, and tropospheric ozone levels in the Park can be high, suggesting that ozone may be causing growth impairment of these plants. The objective of this research was to test the ozone sensitivity of selected conifer species under controlled exposure conditions. Seedlings of three species of conifers, Table Mountain pine (*Pinus pungens*), Virginia pine (*Pinus virginiana*), and eastern hemlock (*Tsuga canadensis*), were exposed to various levels of ozone in open-top chambers for one to three seasons in Great Smoky Mountains National Park in Tennessee, USA. A combination of episodic profiles (1988) and modified ambient exposure regimes (1989–92) were used. Episodic profiles simulated an average 7-day period from a monitoring station in the Park. Treatments used in 1988 were: charcoal-filtered (CF), 1.0× ambient, 2.0× ambient, and ambient air–no chamber (AA). In 1989 a 1.5× ambient treatment was added, and in 1990, additional chambers were made available, allowing a 0.5× ambient treatment to be added. Height, diameter, and foliar injury were measured most years. Exposures were 3 years for Table Mountain pine (1988–90), 3 years for hemlock (1989–91), and 1 and 2 years for three different sets of Virginia pine (1990, 1990–91, and 1992). There were no significant ($p<0.05$) effects of ozone on any biomass fraction for any of the species, except for older needles in Table Mountain and Virginia pine, which decreased with ozone exposure. There were also no changes in biomass allocation patterns among species due to ozone exposure, except for Virginia pine in 1990, which showed an increase in the root:shoot ratio. There was foliar injury (chlorotic mottling) in the higher two treatments (1.0× and 2.0× for Table Mountain and 2.0× for Virginia pine), but high plant-to-plant variability obscured formal statistical significance in many cases. We conclude, at least for growth in the short-term, that seedlings of these three conifer species are insensitive to ambient and elevated levels of ozone, and that current levels of ozone in the Park are probably having minimal impacts on these particular species.

Keywords: Ozone; Conifers; Great Smoky Mountains National Park; Growth; Foliar injury

1. Introduction

Ozone is recognized as the most widespread, and phytotoxic air pollutant in the eastern USA (McLaughlin, 1985; US EPA, 1996; Skelly et al., 1997). Because of this, the US National Park Service is concerned about the potential impacts of ambient and elevated ozone on plants in its parks (Shaver et al., 1994), and continues to support research on ozone exposure–response relationships and bioindicators (Neufeld et al., 1992, 1995; Hildebrand et al., 1996; Chappelka et al., 1997; Kohut et al., 1997; Chappelka and Samuelson, 1998). In the eastern USA, intensive research has been concentrated in three national parks: Great Smoky Mountains National Park (GRSM), which receives long-range transport of pollutants from both the industrialized upper midwest and from states to the south and west,

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☆ This paper is dedicated to the memory of Dr. Jim Weber, a fine scientist and colleague. We will miss his humor, his intellectual contributions to the fields of air pollution and plant ecophysiology, and most of all, his friendship.

most notably Louisiana through Tennessee; Shenandoah National Park (SNP), approximately 480 km to the north, and which comes under the influence of pollutants from the Washington, DC metropolitan airshed; and Acadia National Park (ANP) in Maine, which is subject to pollutant inputs from the New York City–Boston corridor. Visible injury, consistent with known ozone symptoms, has been found on a large number of plants in each of these parks (Neufeld et al., 1992; Hildebrand et al., 1996; Chappelka et al., 1997; Kohut et al., 1997; Chappelka and Samuelson, 1998).

In the mid-1980s, park researchers found that Table Mountain pine (*Pinus pungens* Lambert), a southern Appalachian endemic that is primarily restricted to dry, rocky ridges, displayed chlorotic mottling on needles in the field. These symptoms were similar to those known to be induced on other pine species by exposure to ozone (Berry, 1971; Skelly et al., 1987; Anderson et al., 1988). Mottling has also been noted on eastern white pine (*Pinus strobus* L.) in the south and in ANP (Anderson et al., 1988; Bennett et al., 1994; Kohut et al., 1997), suggesting that these conifers were being stressed from ozone exposure (Benoit et al., 1982), although as Bennett et al. (1994) point out, there is little or no evidence that foliar injury in this species is correlated with growth declines or mortality.

The response of conifers to air pollution is of particular concern, since evergreen species are exposed for a greater number of days each year than their deciduous counterparts, leading to potentially higher ozone doses, and also because these species are of critical ecological importance within most eastern national parks. Although a great deal of research has been devoted to the responses of high elevation spruce–fir forests and commercially important pines of the southern coastal plain and piedmont to ozone and acid rain (Eager and Adams, 1992; Flagler, 1992; Thornton et al., 1994; Flagler et al., 1998), relatively little work has been conducted on the ozone sensitivities of non-commercial conifers native to the southern Appalachians.

Most of the early studies of ozone sensitivity in conifers used acutely high concentrations, and/or square wave exposures. Davis and Wood (1972) exposed 18 species of conifers in growth chambers to high ozone concentrations for short durations (100 ppb for 8 h, 250 ppb for 4 h), grouping species according to whether or not they showed foliar symptoms. Of those species native to the southern Appalachians, eastern hemlock (*Tsuga canadensis* [L.] Carr.), and white pine developed injury after 8 h exposure to 250 ppb, while Virginia pine (*Pinus virginiana* Mill.) showed injury after just 4 h exposure to that concentration. All three species were classified as sensitive based on the development of foliar injury. Further work on Virginia pine showed that younger needles were most sensitive to ozone, while secondary needles and dormant trees exposed in December showed no symptoms (Davis and Wood, 1973). Davis and Wilhour (1976) later modified their rankings of ozone sensitivity for various conifer species, keeping Virginia pine in the sensitive category, but moving hemlock to moderate sensitivity, along with another species, e.g. Pitch pine (*Pinus rigida* Mill.).

Kress and Skelly (1982) exposed Virginia pine seedlings to lower concentrations of ozone in square wave exposures (CF, 50, 100, and 150 ppb) for 6 h day^{-1} for 28 days, using continuously stirred tank reactor chambers. They found no foliar injury or growth effects in any of their treatments.

Wilhour and Neely (1977) exposed a variety of western conifer species to moderate concentrations of ozone (100 ppb for 6 h day^{-1}, 7 day $week^{-1}$ for up to 22 weeks). Only two pine species showed growth reductions related to ozone exposure: ponderosa and western white pines (*Pinus ponderosa* Laws. and *Pinus monticola* Dougl.), with ponderosa pine showing much greater foliar injury than western white pine. The authors concluded that there was no consistent relationship between foliar injury and growth response among the species.

Based on these findings, and the possibility that ozone levels in the eastern USA might be high enough to impact plant growth (Mueller, 1994), we decided to test the ozone sensitivity of several conifer species native to GRSM using an open-top chamber facility located within the Park near Gatlinburg, TN. This was part of a larger project to assess the sensitivities of plants in GRSM to ozone and to develop exposure–response relationships for a variety of species (Neufeld et al., 1992). Three coniferous species common to GRSM were selected for further study: Table Mountain pine, a southern Appalachian endemic (Zobel, 1969), Virginia pine, a common, small- to medium-sized tree of road-cuts, and open disturbed sites, and eastern hemlock, a late successional, shade-tolerant conifer of low- to mid-elevation cove forests (Whittaker, 1956). Our objectives were to assess the response of seedlings of these species to controlled amounts of ozone, and to develop ozone–exposure response curves for growth and biomass accumulation. In addition, we speculate whether ozone might be impacting these species in the field.

2. Materials and methods

2.1. Collection and preparation of plant materials

Open pollinated seeds of Table Mountain pine and eastern hemlock were collected from several sites within the boundaries of GRSM, and then pooled prior to germination. All seeds were stratified for several weeks at 5°C to break dormancy, then planted in 20-cm-diameter pots, using a soil-less mix that insured adequate drainage (Pro-mix, Grace-Sierra Corp., Greenville, SC, USA), and germinated in a greenhouse near the Park. Virginia pine seedlings (wild type) were obtained from a nearby nursery in Morganton, NC, in 1989 and in 1991 These were 1/0 seedlings when placed in the chambers. Both species of pine seedlings received slow-release fertilizer (Osmocote, Sierra Chemical Co., Milpitas, CA, USA) and 15 ppm Mg, Epsom salts (UPS Grade $MgSO_4$) at the beginning of each growing season, whereas the hemlock seedlings were fertilized once a week throughout the growing season with half-strength Peters (N:P:K, 20:20:20) liquid fertilizer. All plants were watered daily to excess to insure good soil moisture conditions.

Table Mountain pine seedlings were moved from the greenhouse to the exposure facility in early May and allowed to acclimate for 10 days before being moved into the chambers. The hemlock were grown on-site, while the Virginia pine were purchased in the autumns of 1989 and 1991 and then transplanted the following springs before being moved into the chambers.

2.2. *Allocation of plants to treatments*

Initial heights and diameters of seedlings of the pines were measured before the beginning of exposure, and seedlings assigned to one of four (1988), five (1989) or six (1991) size categories (this changed according to the number of treatments) based on the statistic: diameter squared times height ($d^2 \times$h). Seedlings within these categories were then randomly assigned to each of the ozone treatments, insuring that the mean sizes among chambers were nearly identical at the start of the experiments. Since the hemlock seedlings were so short initially, they were stratified and distributed to treatments on the basis of stem diameter only. Approximately 10 seedlings per species were allocated to each chamber. For the Virginia pine exposed in 1990, 30 seedlings were allocated initially, with 10 harvested for each of the next two exposure seasons. The remaining 10 seedlings were harvested prior to initiation of the ozone treatments in the spring of 1992 for other purposes, and are not further discussed. Seedlings not harvested in any year were overwintered on-site, and the pots covered with sawdust to minimize frost damage to the roots.

2.3. *Ozone exposures*

In 1988, 7-day ozone profiles were developed from previous year's data at the Look Rock monitoring station in the Park. Treatments imposed that year were: open plots (AA), charcoal-filtered (CF), 1.0×, and 2.0× profiles. In subsequent years, modified ambient treatments were used instead of synthesized profiles, in order to better link ozone dynamics to weather conditions, and to make our protocols more comparable to other studies being conducted at that time. In 1989, these included AA, CF, 1.0×, 1.5×, and 2.0× treatments. In 1990, after enlargement of the exposure system from 9 to 15 chambers, a 0.5× treatment was added. There were three replicate chambers for each treatment in 1988; two in 1989, except for the 2.0× and AA treatments, which had three, while in 1990–92 there were three replicates for all treatments (see Neufeld et al., 1992, for more details about the exposure system).

Seedlings were exposed 7 day week^{-1}, 24 h day^{-1}. Ozone was produced by an electric spark discharge generator (Ozone Research Co., Phoenix, AZ, USA), supplied with air (1988) and from 1989 to 1992, liquid oxygen. Ozone was dispensed to the chambers under constant flow conditions using rotameters. A Campbell 21× data logger (Campbell Scientific Inc., Logan, UT, USA) adjusted the voltage output of the ozone generator four to five times per hour to control the amount of ozone dispensed, based on monitor readings from the AA plots.

Ozone concentrations for all plots were monitored by two (1988–89) or three (1990–92) time-shared TECO Model 49 analyzers (Thermo Environmental Instruments Inc., Franklin, MA, USA). Air from each chamber and plot was continuously pulled through a manifold system and analyzed four to five times per hour for ozone concentrations. Teflon tubing and filters were used in all parts of the system, and periodic checks consistently showed less than 5% line losses over the seasons. Analyzers were calibrated weekly and audited quarterly by the State of Tennessee Division of Health and Environment and Air Resources Specialists Inc. (Ft. Collins, CO, USA). In all cases, monitors were within established US EPA quality control and assurance guidelines.

Standard open-top chambers (3 m diameter) were used (Heagle et al., 1973). Chambers did not have frustra or raincaps until 1990. The chambers were covered with 50% shade cloth only in 1988. The incorporation of frustra has been shown to reduce ambient air intrusions and to increase uniformity of ozone concentrations within the chamber (Davis and Rogers, 1980). The raincaps helped us to avoid potentially damaging high winds and rain. Although herbivory was not a problem on the species in this study, netting was used to minimize herbivore damage on other species in the chambers. The exposure system was operative for over 90% of the time every season, and up to 95% in 1989.

Exposure periods are provided in Table 1. The total cumulative exposures (12-h exposures, 08.00–20.00) are also shown for comparative purposes. The SUM06 is the seasonally adjusted (see Lee et al., 1991, for a description of how exposure indices are adjusted for missing values) sum of the hourly mean concentrations whose values equal or exceed 0.060 ppm (Lee et al., 1988) while the AOT40 is the adjusted sum of the

Table 1
Exposure periods, exposure indices[a] (ppm×h), and number of hours with ozone greater than 0.100 ppm for species exposed[b]

Species	Date		Ozone treatment																	
	Year	Exposed	AA			CF			0.5×			1.0×			1.5×			2.0×		
			Sum06	AOT40	h > 0.100 ppm	Sum06	AOT40	h > 0.100 ppm	Sum06	AOT40	h > 0.100 ppm	Sum06	AOT40	h > 0.100 ppm	Sum06	AOT40	h > 0.100 ppm	Sum06	AOT40	h > 0.100 ppm
Eastern hemlock	1989	16 June–28 Sept.	3.5	3.1	0	0.1	0.1	0.5	–	–	–	2.1	2.2	0.5	14.8	8.9	14.8	28.5	17.1	78.3
	1990	30 June–25 Sept.	2.2	2.4	1.1	0.1	0.1	0	–	–	–	0.9	1.6	0	14.2	9.8	12.2	32.9	19.3	52.9
	1991	22 May–8 Oct.	0.9	1.6	0	0.0	0.0	0	–	–	–	0.5	1.3	0	19.9	13.3	7.6	46.7	27.5	86.8
		Total	6.6	7.1	1.1	0.2	0.2	0.5	–	–	–	3.5	5.1	0.5	48.9	32.0	34.6	108.1	63.9	218.0
Table Mountain pine	1988	1 July– 24 Aug.	5.3	3.8	0	0.0	0.0	0	–	–	–	20.8	11.9	5.4	–	–	–	66.7	42.3	343.2
	1989	15 June–28 Sept.	3.5	3.2	0	0.1	0.1	0.5	–	–	–	2.1	2.2	0.5	–	–	–	28.6	17.2	78.5
	1990	30 June–22 Aug.	1.9	1.8	1.2	0.1	0.1	0	–	–	–	0.8	1.2	0	–	–	–	21.1	12.2	35.0
		Total	10.7	8.8	1.2	0.2	0.2	0.5	–	–	–	23.7	15.3	5.9	–	–	–	116.4	71.7	456.7
Virginia pine	1990	30 June–27 Sept.	2.2	2.4	1.1	0.1	0.1	0	0.1	0.1	0	0.9	1.6	0	14.2	9.8	12.1	32.8	19.3	52.7
Virginia pine[c]	1991	6 May–23 Sept.	0.9	2.6	0	0.0	0.0	0	0.3	0.2	1.1	0.6	1.4	0	20.7	13.7	7.7	47.9	27.1	90.1
Virginia pine	1992	4 May–9 Oct.	2.9	3.8	0	0.1	0.1	0	0.0	0.0	0	2.0	2.4	0	24.6	15.5	17.6	56.2	34.4	133.8

[a] Sum06, cumulative sum of hourly averages 60 ppb or greater (08.00–20.00); AOT40, cumulative sum of the difference in hourly averages between 40 ppb and higher (08.00–20.00).
[b] 08.00–20.00.
[c] Add 1990 exposures to get total exposure; these plants were exposed in 1990 and 1991.

differences in hourly concentrations between 0.040 ppm and higher values. The number of hours above 0.100 ppm is also provided in Table 1.

2.4. Analysis of plant material

At approximately biweekly intervals, plants were measured for height and diameter. At the conclusion of a growing season, those plants to be harvested were divided into needles (each age class handled separately), stems, branches, and roots. For Virginia pine, stem, needle and branch biomass were further subdivided by whorl within year (there were up to seven distinct whorls on some plants). Projected needle area was determined with a Li-Cor 3000 leaf area meter (Li-Cor, Inc., Lincoln, NE, USA) calibrated against a US National Bureau of Standards certified traceable disk. All biomass fractions were dried to constant weight at 55–60°C, and weighed to the nearest 0.01 g. Table Mountain pine and eastern hemlock were harvested only once at the end of their 3-year exposure, thus sample sizes were approximately 10 seedlings per chamber.

Foliar injury symptoms were noted when seen, and quantified at the end of a growing season. Measurements were made in 1988 and 1989 for Table Mountain pine, 1989–91 for hemlock, and 1990 and 1992 for Virginia pine. Two to three needles per age class on the main stem were observed each time, and the percent length of chlorotic mottling, tip-burn and necrosis noted. Because significant effects for tip-burn and necrosis were never found, we have excluded them from further discussion.

2.5. Quality assurance and quality control

Data were quality checked and assured using protocols developed by the US EPA (Evans and Dougherty, 1986). Precision and accuracy checks were made on all instruments, both before and after measurements were taken. Reliability was checked by re-measuring 5% of the samples. All data entered into the computer were checked against the original data to reduce the chances of data entry errors.

2.6. Statistical analyses

Biomass and growth data were subjected to analysis of variance (ANOVA) using SAS, to test for ozone treatment effects (SAS Inst. Inc., 1989). The ANOVA model included terms for ozone, chamber within ozone, and plants within chamber. The main effect of ozone was tested using the mean square error for chamber within ozone. ANOVA was also used to test for differences between the AA and 1.0× treatments. Post-ANOVA analyses included orthogonal polynomial contrasts to test for linear and quadratic treatment effects. The general lack of significant ozone effects, however, precluded any attempts at fitting regressions to the data, as would normally be done when the treatment factor consists of multiple levels of a single, ordinal variable. A planned contrast (two-sided t-test) was used to compare the AA and 1.0× treatments, while one-sided Bonferroni tests were used to compare treatment means (Hochberg and Tamhane, 1988). We used one-sided tests because we assumed ozone would only cause decreases in biomass, and only increases in foliar injury. This means that the critical p values used to determine significance were divided by the number of pairs of means being compared (e.g. if 0.05 is the critical p value, and there are five treatment means, then the critical p value would be $p/10 = 0.005$, since 10 pairs of means could be compared in this situation).

Percent chlorotic mottle violated the assumption of constant variance. Several transformations, including the square root and logarithmic transformations were considered, but they did not alleviate the problem of heterogeneity of variance. Instead, weighted ANOVA was used to test for treatment differences in chlorotic mottle using weights proportional to the reciprocal of the plant-to-plant variation, averaged across replicate chambers. The same post hoc tests as for biomass were used for foliar injury.

3. Results

3.1. Ozone exposures

Ozone treatments in all years were substantially different from each other (Table 1), due both to varying exposure durations, and yearly variability in ozone conditions. Ambient values for the AOT40 (which is the statistic reported in this section, and which is highly correlated with the SUM06) ranged from 1.6 ppm×h for hemlock in 1991, to 3.8 ppm×h for Table Mountain pine in 1988, one of the highest ozone years on record. Note that in 1988, profiles were synthesized from data within GRSM, and repeated on a 7-day cycle, which partly explains the differences in exposures between that year and the other years. Ambient air was also used to produce the ozone, instead of pure oxygen, which can result in the formation of nitrous oxides, but which apparently did not cause any toxic effects that year. In comparison, 1989 and 1991 were low ozone years, and the AOT40s reflect this by being fairly low (Table 1). Of the three species considered in this paper, hemlock was exposed the most number of days; over a 3-year period (1989–91) hemlock received ozone on 333 days, with a cumulative 1.0× exposure of 5.1 ppm×h (63.9 ppm×h in the 2.0× treatment). Table Mountain pine was exposed for a total of 215 days over 3 years (1988–90), and received cumulative exposures of 15.3 and 71.7

ppm×h in the 1.0× and 2.0× treatments, respectively. In 1988 alone, this species received an exposure of 11.9 ppm×h, nearly 78% of its total cumulative exposure. Single season exposures for Virginia pine ranged from 1.4 ppm×h in 1991 to 2.4 ppm×h in 1992, while those seedlings exposed over two seasons (1990–91) received 3.0 ppm×h in the 1.0× treatment and 46.4 ppm×h in the 2.0× treatment.

The most notable effect of the treatments was a sharp increase in the number of hours of high concentrations in the elevated ozone treatments. For example, in 1988, Table Mountain pine experienced only 5 h >0.100 ppm at ambient (<1% of the daylight hours), but 343 h in the 2.0× treatment (52% of the daylight hours). As noted above, 1988 was an exceptional ozone year, since in all the other years of these studies the number of hours above 100 in the 2.0× treatment averaged just 5–7%, compared to either zero or less than 2% in the 1.0× treatment. Ambient ozone loads varied from year to year, with the average daylight hourly concentrations much higher in 1988 (0.035 ppm) than any of the following years (range, 0.025 ppm in 1991 for hemlock, to 0.029 ppm for Table Mountain pine in 1990).

The start and stop dates varied from season to season, and that, coupled with the different ozone loads each year, caused total exposures in the 1.0× treatment to range over an order of magnitude, from 15.3 ppm×h for Table Mountain pine, to 5.1 ppm×h for hemlock, to less than 3 ppm×h for any of the Virginia pine seedling sets (Table 1).

3.2. *Biomass effects*

3.2.1. *AA versus 1.0×—all species*

Throughout the duration of the study, chamber effects on biomass and growth were negligible for all species. No significant differences ($p>0.05$) were found between the AA and 1.0× treatments for any parameter measured (data not shown).

3.2.2. *Table Mountain pine*

There were no significant effects ($p>0.05$) for height or diameter in any season, so only the data for the last year are shown (Fig. 1). There were also no ozone effects on any biomass fraction, even after 3 years of exposure (Fig. 2), except on the oldest needles (those formed in 1988), which significantly decreased in the 2.0× treatment ($p=0.0097$).

3.2.3. *Eastern hemlock*

After 3 years of exposure, most biomass fractions showed no significant treatment effects (Fig. 3), nor were height or diameter affected (Fig. 1). A few parameters (root:shoot ratio, leaf area and weight of current year needles) did have significant or marginally significant treatment effects, but the patterns were not correlated with increasing ozone. Instead, most of these treatment effects resulted from unusually high means in the 1.5× treatment.

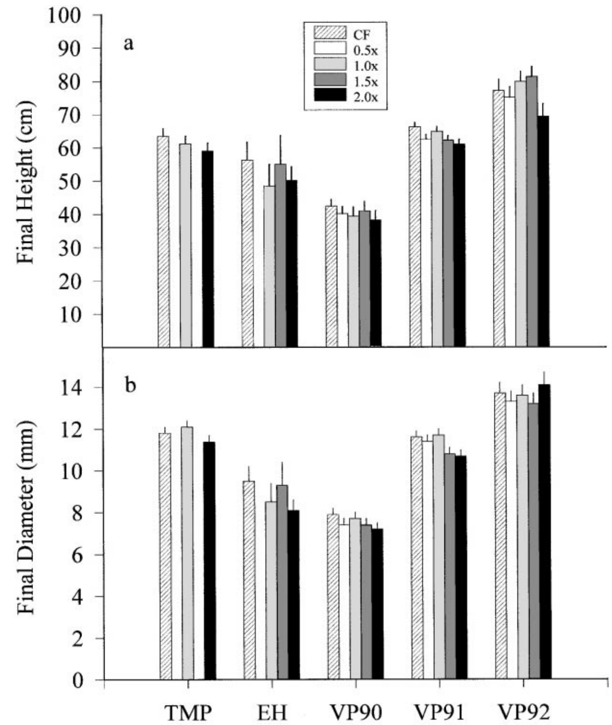

Fig. 1. (a) Final heights and (b) diameters ±SE for each species after exposure to ozone at the open-top chamber facility in Great Smoky Mountains National Park (GRSM). $n=2$–3. Species key: TMP, Table Mountain pine; EH, Eastern hemlock; VP90, Virginia pine exposed in 1990; VP91, Virginia pine exposed for two seasons (1990 and 1991); VP92, Virginia pine exposed in 1992.

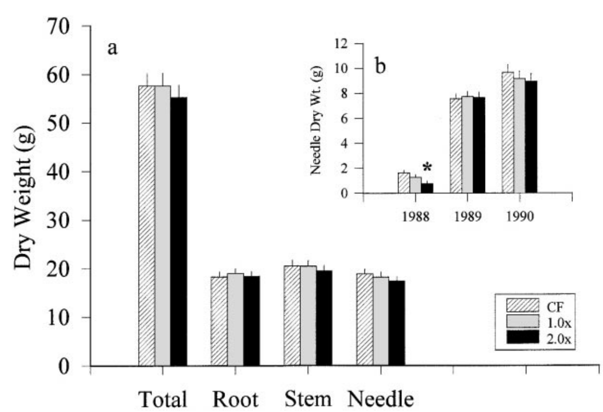

Fig. 2. Biomass of Table Mountain pine after (a) three seasons of exposure to ozone, and (b) needle biomass separated by year of formation. All plants exposed at open-top chamber facility in Great Smoky Mountains National Park (GRSM). Values are mean±SE, $n=2$–3. Asterisk indicates treatment mean is significantly different ($p<0.05$) from CF.

3.2.4. *Virginia pine*

ANOVA revealed no significant treatment effects on height or diameter, or on any biomass fraction (Figs. 1

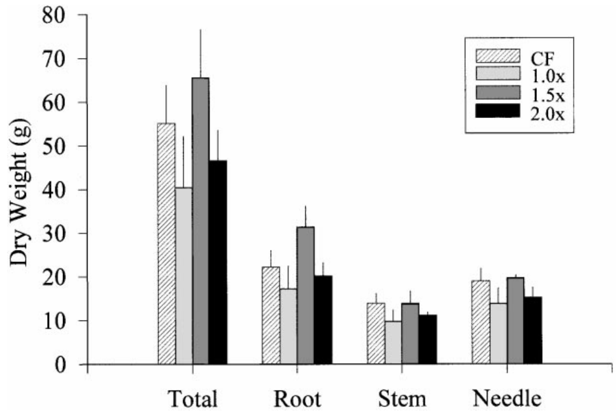

Fig. 3. Biomass of hemlock after three seasons of exposure to ozone at open-top chamber facility in Great Smoky Mountains National Park (GRSM). Values are mean ± SE, $n = 2$–3.

and 4), except for a trend toward lower total needle weight in the 2.0× treatment ($p = 0.0541$) in the 1990 exposure season. There was a significant increase in the root:shoot ratio ($p = 0.0359$), resulting from a proportionally greater loss of needle weight than root weight. Linear effects were significant for total stem weight, which decreased in both the 1.5× and 2.0× treatments ($p = 0.0472$ for linear effect), root:shoot ratio ($p = 0.0024$) and first flush stem weight and 1989 needle weight, which showed decreases primarily in just the 2.0× treatment ($p = 0.0298$ and 0.0089, respectively). The same patterns held true after the second year of exposure in 1991 (Fig. 4), although the treatment effect for 1-year-old needle biomass (1990 needles) was statistically significant ($p = 0.0339$). Post hoc comparisons for these needles showed that the 2.0× treatment was significantly lower than the CF treatment ($p = 0.0033$). For seedlings exposed in 1992, the trends were similar (Fig. 4): no significant treatment effects were found for any variable except 1-year-old needle biomass ($p = 0.0557$), which decreased in the 2.0× treatment. Two-year-old needles (those formed in 1990) did not show a response to ozone ($p = 0.1322$).

3.3. *Foliar injury patterns*

3.3.1. *AA versus 1.0×*

Percent chlorotic mottling in the 1.0× treatments was higher than for the AA plots for Table Mountain pine in 1989. This was true for both age classes of needles (15 ± 5% vs. 3 ± 5%, current year; 15 ± 5% vs. 5 ± 5%, 1 year old). No differences were noted in the 1988 census (3 ± 1% vs. 3 ± 1%, current year; 4 ± 1% vs. 5 ± 1%, 1 year old). Virginia pine in 1990 showed significantly less injury in the 1.0× treatment compared to the AA plots, but only for current year needles (17 ± 6% vs. 7 ± 2%). No differences were seen for any age class of Virginia pine needles in 1992 (all values < 3%). Hemlock never showed any foliar symptoms.

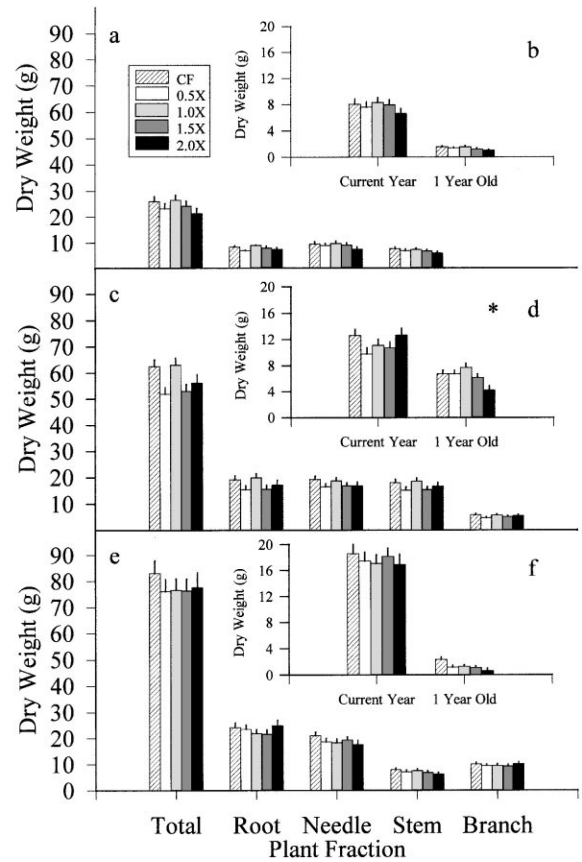

Fig. 4. Biomass of Virginia pine exposed in (a) 1990, with (b) needle biomass separated by year of formation; (c) biomass of Virginia pine exposed in 1990 and 1991, with (d) needle biomass separated by year of formation, and (e) Virginia pine exposed in 1992, with (f) needle biomass separated by year of formation. All plants exposed at open-top chamber facility in Great Smoky Mountains National Park (GRSM). Values are mean ± SE, $n = 3$. Asterisk indicates treatment mean is significantly different ($p < 0.05$) from CF.

3.3.2. *Table Mountain pine*

Table Mountain pine exhibited greater needle mottling in the 2.0× treatment in 1988 and in 1989 than in the CF, AA, or 1.0× treatments (Fig. 5). However, the great variability among plants and among needles within plants prevented the 2.0× treatment from being statistically different in 1989. The 1.0× treatment was statistically different at the 0.10 level of significance, and then only for current year needles. There was a statistically detectable difference between the CF and 1.0× treatment in current year needles in 1988, but given the precision of foliar injury surveys, the magnitude of this difference is biologically insignificant. The trend in both years is clearly an ozone effect, however. In 1989 no needles had greater than 5% mottling in the CF treatment, while all higher values were restricted to the 1.0× and 2.0× treatments.

3.3.3. *Virginia pine*

In 1990 both current and 1-year needles in the 2.0× treatment had statistically greater injury than those in

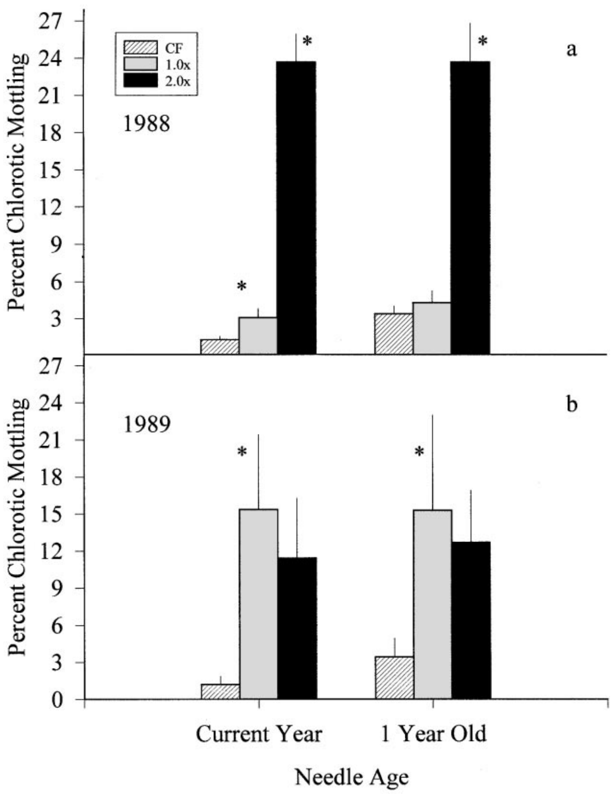

Fig. 5. Ozone effects on percent chlorotic mottling for Table Mountain pine needles of different ages in (a) 1988 and (b) 1989. All plants exposed at open-top chamber facility in Great Smoky Mountains National Park (GRSM). Values are mean ± SE, $n = 2$–3. Asterisk indicates treatment mean is significantly different from CF ($p < 0.05$ for 1988, $p < 0.10$ for 1989).

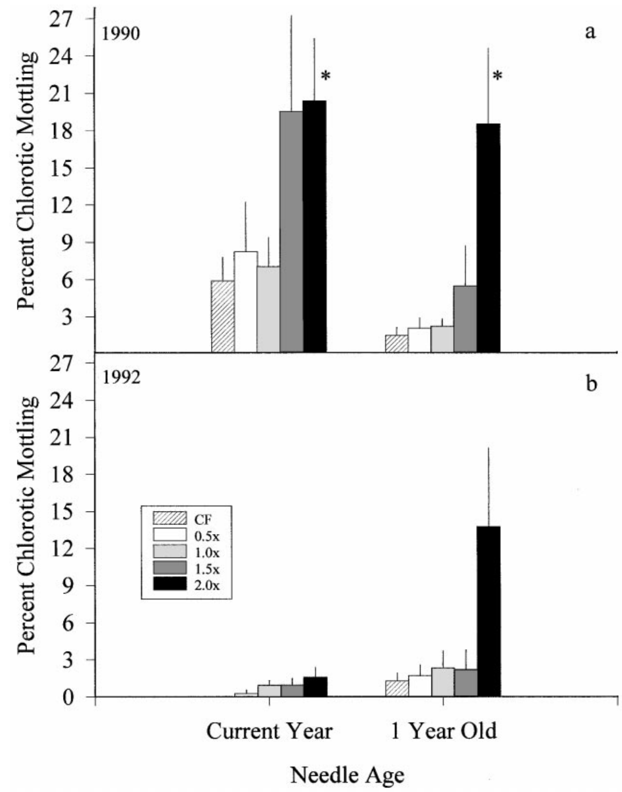

Fig. 6. Ozone effects on percent chlorotic mottling for Virginia pine needles of different ages in (a) 1990 and (b) 1992. All plants exposed at open-top chamber facility in Great Smoky Mountains National Park (GRSM). Values are mean ± SE, $n = 3$. Asterisk indicates treatment mean is significantly different from CF ($p < 0.10$).

the CF treatments (and for 1-year-old needles also the 0.5x treatment), but only at the 0.10 level of significance (Fig. 6). There were no statistically significant differences in injury among treatments in 1992 ($p > 0.10$). The large differences seen for 1-year-old needles between the lower treatments and the 2.0× treatment were not significant due to the large standard error in the 2.0× treatment. Injury was greater in the 1990 seedlings than the 1992 seedlings, despite the fact that ozone exposure in the 2.0× treatment was approximately 79% higher in 1992 than 1990 (AOT40 of 34.408 vs. 19.258 ppm×h, respectively).

4. Discussion

None of the species analyzed in this study exhibited statistically significant responses to ozone in terms of total biomass accumulation, height or diameter growth even when exposed for three consecutive years. However, both Table Mountain pine and Virginia pine did show a pattern of increased foliar injury with more ozone exposure, suggesting that these two species can be affected to some degree if the ozone gets high enough. Only the two higher treatments exceeded the AOT40 of 10.5 ppm, which Fuhrer et al. (1997) have suggested is the critical exposure for detectable growth effects for tree species in Europe. The occasional discrepancies in foliar injury between the AA and 1.0× treatments are difficult to explain, but certainly some sort of chamber effect, and/or misdiagnoses could be at fault here (Hacker and Neufeld, 1992). In any event, when the AA plots are omitted from the statistical analyses, a clear ozone effect on chlorotic mottling is evident, even if the results are not always significant in a formal statistical sense.

In several cases, foliar injury occurred with ozone exposures that were lower than those in a previous year that did not elicit injury. For example, Table Mountain pine showed injury in 1989 at an AOT40 of 2.1 ppm×h, whereas in 1988 an exposure of over 20 ppm×h did not cause visible injury. For Virginia pine, similar amounts of injury were obtained in the 2.0× treatment in both 1990 and 1992, despite AOT40 exposures of 33 and 56 ppm×h, respectively. With regards to the Table Mountain pine, the differences might have been due to the different exposure regimes employed from one year to the next (simulated vs. modified ambient), while for the Virginia pine, the situation is less clear. Possibly interactions with weather played a role (1992 was a notably wet year) and/or these plants responded with some maximal amount of injury for the levels of ozone encountered.

It is not surprising, though, that foliar injury occurred in the absence of a growth response. As pointed out by Chappelka and Chevone (1992), foliar injury alone is not a sufficient predictor of growth responses, particularly for trees. But the lack of an effect on growth is still interesting, considering that the ozone exposure protocols tended to substantially increase the incidences of very high peak concentrations (Table 1), which are thought important in causing foliar injury and reducing growth (Heck and Tingey, 1971; Musselman et al., 1994).

There are several possible reasons why we did not detect a growth effect in these studies. First, high plant-to-plant and/or chamber-to-chamber variation reduced the power of our statistical tests. Genetic variation may have been a factor, but our experiments were not designed to consider this possibility. The same reasons also probably apply to the general lack of statistical significance for foliar injury. Second, we may not have exposed our seedlings long enough to detect growth inhibitions. Slow-growing woody species may require many years of exposure to accumulate statistically detectable treatment differences (Hogsett et al., 1995). For example, even after many years of exposure to relatively high ambient ozone concentrations, growth reductions could not be detected in ponderosa pine of the Sierra Nevada mountains of the western USA (Peterson and Arbaugh, 1988; Peterson et al., 1991).

The species observed in this study appear to be among the most tolerant tree species to ozone of those tested in GRSM (see Neufeld et al., 1992, for a complete list of plants exposed during the years 1988–92). Although sensitivity and/or tolerance mechanisms for trees are not fully understood at present (Taylor et al., 1994), we can speculate as to why the particular species in this study have low sensitivity to ozone. Table Mountain pine is found mainly on dry, rocky ridges in the southern Appalachian mountains, and in areas subject to frequent fire (Williams and Johnson, 1990, 1992). These habitats are prone to drought in the summer, during which the trees may temporarily close their stomata to avoid water stress. Since ozone is absorbed primarily through stomata this would lower the dose received, and lessen the impact on the plant. Temple et al. (1992, 1993) showed that drought greatly reduced foliar symptoms on ponderosa pine. We have noticed that seasonal development of foliar symptoms in hardwoods in GRSM is hindered by drought, and that symptoms often appear immediately after rain events (personal observations of the authors). The lack of a detectable effect on Table Mountain pine in this study, even after 3 years of exposure and adequate watering (which would minimize stomatal closure due to water stress), suggests that this species may *avoid* ozone uptake via an inherently low stomatal conductance to water vapor (and hence to ozone). However, the authors could find no data in the literature on stomatal conductance for this species. Alternatively, it may possess a high physiological *tolerance* to ozone absorbed into the needles, or some combination of these two (Tingey and Taylor, 1982). In contrast, McLaughlin and Downing (1996) have found evidence that ambient ozone exposures may prevent normal stomatal closure in loblolly pine (*Pinus taeda* L.), possibly exacerbating drought stress and reducing growth.

Although we did find foliar injury in this study, it may not translate into an effect on growth. Studies have shown that the loss of older foliage (as seen in this study for the Table Mountain and Virginia pines) often has little effect on growth responses, presumably because of the lower physiological activity of these needles, and because the younger needles compensate physiologically (Linzon, 1958; Beyers et al., 1992). The low expression of ozone injury by the seedlings in this study, and the fact that adult conifers generally have lower stomatal conductances than their seedlings (Thornton et al., 1994; Yoder et al., 1994), suggests that mature trees in the field may be even less sensitive to ambient ozone than seedlings, especially if they experience drought stress during the growing season.

Eastern hemlock may avoid ozone uptake also because of low stomatal conductance, but this may be more a reflection of the successional status of this species than habitat conditions: late successional plants often have reduced rates of gas exchange compared to early successional plants (Harkov and Brennan, 1982). Thus, all three of these relatively slow growing species may share a common avoidance mechanism of low stomatal conductance, which results in low ozone uptake, giving needles time to detoxify and repair any damage that might occur (Weber et al., 1993, 1994).

In conclusion, none of these common conifer species appears to be sensitive (at least in the seedling stage) to ambient exposures of ozone that typically occur in GRSM and, in fact, are remarkably insensitive to 2.0× times ambient in most cases. We caution, however, that detectable effects may only become apparent over very long time periods (i.e. decades) because of the relatively slow growth rates of these species: ozone will continue to be a pollutant of great concern in the long term (Skelly et al., 1997).

There are, however, important and consequential short-term threats to each of these species that, in contrast, are biological in origin. Lack of fire and subsequent suppression of regeneration by competing plants threaten Table Mountain and Virginia pine, whereas hemlock is threatened by the hemlock woolly adelgid (*Adelges tsugae*), a recently introduced pest from Europe (Orwig and Foster, 1998).

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