

# Sensitivity of seedlings of black cherry (*Prunus serotina* Ehrh.) to ozone in Great Smoky Mountains National Park

## I. Exposure–response curves for biomass

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### SUMMARY

The response of seedlings of black cherry (*Prunus serotina* Ehrh.) to ozone was evaluated in Great Smoky Mountains National Park using open-top chambers during the growing seasons of 1989 and 1992. Two separate sets of seedlings were each exposed to various concentrations of ozone (charcoal-filtered; 0.5 × (not used in 1989), 1.0 ×, 1.5 ×, and 2.0 × modified ambient) in two different seasons. Seasonal indices of exposure (SUM00, SUM06 and AOT40) for the 1.0 × treatments were 39.2, 1.9, and 1.62 ppm h respectively, in 1989, and 63.1, 0.9, and 0.78 ppm h, respectively, in 1992. No significant chamber effects were noted, except for reduced height growth in open plots compared with 1.0 × chambers in 1992. In both years, the 2.0 × treatment reduced total, leaf, root, and shoot + root biomass, although some of these changes were only marginally significant in 1992. Stem biomass was significantly reduced in 1989, but not 1992. Leaf area, count and weight were all highly correlated, and showed significant reductions in both years. The leaf area ratio (leaf area/total weight) was reduced in 1989, but not in 1992. Height was not affected by ozone, but diameter was reduced only in 1989. Chamber-to-chamber variation for biomass and leaf variates was greater in 1992, and as a result, significance levels were lower. Weibull functions were fitted to chamber means, and showed significant near-linear declines for most components when log-transformed data were plotted against the SUM06 and AOT40 indices. Individual Weibull models for the 1989 and 1992 data sets, and combined models over both years, were developed. Combined models were adequate for describing ozone responses for all biomass components, as determined by the likelihood ratio test. The data show that the two years of exposure produced similar, but not identical effects, despite large differences in initial size of the seedlings and in seasonal ozone dynamics. Leaf and root biomass were most sensitive to ozone (as determined by the slope of decrease with increasing SUM06), whereas stem biomass was least sensitive. Black cherry seedlings are shown to be among the most sensitive to elevated ozone of the 21 tree species examined to date in Great Smoky Mountains National Park.

Key words: *Prunus serotina*, ozone, growth, Great Smoky Mountains National Park, Weibull response function.

### INTRODUCTION

The National Park System in the United States is responsible for protecting the biological resources within its boundaries, as required by the Organic Act

of 1916 (Shaver, Tonnessen & Maniero, 1994). In 1977 Congress amended the Clean Air Act to protect ecologically important areas, called Class I areas, from further deterioration of air quality. Great Smoky Mountains National Park (GRSM), along with over 150 other areas, have been designated Class I airsheds, and are to receive the highest priority in terms of protecting air quality. Further congressional action established the concept of 'air

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quality related values' which must be protected from additional increments in air pollutant loads. In response to these requirements, the Park Service undertook, beginning in 1987, an extensive study of the effects of air pollution, and in particular, ozone, on the growth and appearance of plants in several national parks, including GRSM (for more details of the history of these investigations, see Shaver *et al.*, 1994). Over a period of 6 yr, from 1987 to 1992, over 90 species of plants in GRSM were reported with ozone-like stipple in the field, and over 35 were exposed in open-top chambers to verify that those symptoms were indeed a result of ozone (Neufeld *et al.* 1992). During the course of those investigations, evidence accumulated that black cherry (*Prunus serotina* Ehrh.) might be very sensitive to ozone, and more detailed studies were undertaken on this ecologically important species.

Black cherry is an important member of many hardwood forest types in North America, extending westward to the plains, and southward into Mexico and Central America (Marquis, 1990). In western Pennsylvania, Maryland, New York and West Virginia, it is a major component of the Cherry-Maple forest type (Eyre, 1980), reaching its greatest size on mesic slopes of the Alleghany and Appalachian Mountains from West Virginia down to Georgia (Sargeant, 1965). In Pennsylvania, it is both ecologically and economically important because of the value of its fruits to wildlife and its wood for furniture (Marquis, 1990). Black cherry is also widespread in Great Smoky Mountains National Park (GRSM), and at its greatest abundance, comprises 1.5% of the stem density in mid-elevation hardwood forests (Whittaker, 1956). It is classified as shade intolerant, and will succumb to shade after prolonged periods in the understorey (Marquis, 1990). Its seeds, however, can remain dormant in the soil for several years and, as a result, it can quickly regenerate in areas that lose their forest cover (Marquis, 1990).

Black cherry has been found previously to be sensitive to ambient ozone, as shown by visible foliar injury (Davis, Umbach & Coppolino, 1981; Renfro, 1989; Long & Davis, 1991). Injury symptoms have been found on black cherry throughout most of its native range, from Pennsylvania in the east (Wood *et al.* 1982; Davis & Skelly, 1992; Simini *et al.*, 1992) south into Great Smoky Mountains National Park (Neufeld *et al.*, 1992), and in Mexico (De Lourdes DeBauer, pers. comm.).

Using open-top chambers, Simini *et al.* (1992) reported increased foliar stipple on black cherry exposed to 40, 60 and 95% of ambient ozone, compared with charcoal-filtered seedlings. They found a positive correlation between ozone exposure and foliar stipple, but no detectable growth effects. Long & Davis (1991) sprayed black cherry with the antioxidant ethylenediurea (EDU), and provided

convincing evidence that the symptoms noted by Simini *et al.* (1992) were most likely to be caused by ozone. In addition, Davis & Skelly (1992) found increased stipple on older leaves of black cherry after exposure to 75 ppb ozone in controlled environment chambers, further supporting the conclusions of Simini *et al.* (1992) that the symptoms observed in the field were indeed a result of ozone. In contrast to Simini *et al.* (1992), Long & Davis (1991) noted that after 4 yr growth in the field, total above-ground dry weight in black cherry trees treated with EDU was 47% greater than those not treated, which they attributed to the effects of ambient ozone.

The objectives of this study were to develop exposure-response curves for black cherry seedlings exposed to ozone in GRSM. We report the results of two separate years of exposure to ozone on two different sets of black cherry seedlings. The studies were made in 1989 and 1992 using the open-top chamber exposure facility located at Uplands Field Research Laboratory, GRSM.

We have compared the results of the two years and have derived ozone exposure-response functions common to both sets of seedlings using both the SUM06 and AOT40 indices (see Methods section for index definitions). Research has shown greater effects on plant growth responses when ozone concentrations increase or when plants are exposed for longer duration (Hogsett, Tingey & Lee, 1988; Musselman, McCool & Lefohn, 1994). Data reported in the literature suggest that higher hourly ozone concentrations are more important than mid- and low-range concentrations and that ozone effects are cumulative. Exposure indices that relate well with plant response should cumulate the hourly concentrations and give greater weight to peak concentrations (Lee, Tingey & Hogsett, 1988; Lefohn, Laurence & Kohut, 1988). Several retrospective studies favoured the peak-weighted, cumulative indices, including the SUM06 index (Lee *et al.*, 1988; Olszyk *et al.*, 1993) and the W126 index (Lefohn *et al.*, 1988) based on better statistical fits to plant biomass. The SUM06 index was selected because it gives the best fits for a large variety of exposure situations, is highly correlated with the W126 index, and has values near zero for pristine areas. We also include the AOT40 index for comparison, as this index has recently been adopted by the UN/ECE and is used extensively in European studies (Fuhrer & Acherman, 1994). Foliar injury and CO<sub>2</sub>-exchange effects will be the subjects of a second paper in this series.

#### MATERIALS AND METHODS

Seeds from several open-pollinated trees growing in GRSM were collected during the autumn of 1988 and 1991, stratified at 4 °C in a moist sand-and-peat medium for at least 90 d to relieve seed dormancy,

and then planted in 20 cm pots (2.8 l volume) filled with a 2:1 (v:v) mixture of bark and Promix® (a soilless potting mix, Grace-Sierra Corp., Greenville, SC). Seeds for the 1992 study were not necessarily obtained from the same mother trees as those used in 1989, but were from the same collection areas in GRSM. Seedlings received  $\frac{1}{2}$  strength Peters® (N:P:K, 20:20:20) liquid fertilizer once a week after germination and throughout the growing season. All plants were watered daily to field capacity, and when fertilized. Benlate® and Diazanone® were used infrequently (no more than five times during a season) to control mildew and insect pests, respectively. Plants were always sprayed in the evening to avoid leaf burn. Although we do not know if the Benlate exerted any antioxidant effects on the black cherry seedlings, there were highly significant effects of ozone which possibly would have been even greater without spraying. More importantly, most of the seedlings would have suffered severe damage as a result of powdery mildew infection had they not been sprayed, and thus we feel our precautions were appropriate.

In 1989, initial heights and diameters of seedlings were measured before the beginning of exposure, and seedlings were assigned to one of five size categories based on the statistic diameter squared times height ( $d^2h$ ). Seedlings within these five categories were then randomly assigned to each of five ozone treatments: open plots, charcoal-filtered (CF),  $1.0\times$ ,  $1.5\times$  and  $2.0\times$  ambient. Ten seedlings were allocated to each plot location, with one seedling in reserve in case of mortality. The initial mean heights and diameters among treatments were not significantly different ( $P > 0.05$ ) at the beginning of the experiment. In 1992, an additional treatment of  $0.5\times$  ambient was added. Because the exposures started later in 1989, seedlings that year were larger at the start of the experiment than those in 1992. Mean initial heights and diameters were 63 cm and 5.6 mm, respectively, in 1989, and 5.2 cm and  $< 1.5$  mm, respectively, in 1992.

Seedlings were exposed  $7\text{ d wk}^{-1}$ ,  $24\text{ h d}^{-1}$  (see Neufeld *et al.* 1992 for more details about the exposure system). Ozone was produced by an electric spark discharge generator (Ozone Research Company, Phoenix, AZ), supplied with pure oxygen. Ozone was dispensed to the chambers under constant flow conditions using rotameters. A Campbell 21  $\times$  datalogger (Campbell Scientific, Inc., Logan, UT) adjusted the voltage output of the ozone generator five times per hour to control the amount of ozone dispensed, based on monitor readings from the open ambient plots.

Ozone concentrations for all the plots were monitored by three time-shared TECO Model 49 analysers (Thermo Environmental Instruments, Inc., Franklin, MA). Air from each chamber and plot was continuously pulled through a manifold

system and analysed 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 season. Analysers were calibrated weekly and audited quarterly by the State of Tennessee Division of Health and Environment and Air Resource Specialists, Inc. (Ft. Collins, CO). In all cases, monitors were within established U.S. EPA quality control and assurance guidelines.

Exposures ran from 14 June to 28 August in 1989, a total of 76 d, and from 20 May to 6 October in 1992, a total of 140 d. Table 1 shows cumulative ozone exposures (SUM00, SUM06 and AOT40) for each chamber, by season. SUM00 values, as detailed in Lee *et al.* (1988), are calculated by summing the hourly averages from the first day of exposure to the last, whereas SUM06 values sum those concentrations  $\geq 60$  ppb. The AOT40 is the sum of the differences between 40 ppb and actual hourly values. The AOT40 gives proportionally more weight to higher concentrations than does the SUM06.

In 1989, each treatment was replicated twice, with the exception of the  $2.0\times$  and open-plot treatments, which had three replications each. In 1992, more chambers were added so that all treatments had three chamber replications.

Standard open-top chambers (3.0 m diameter) were used (Heagle, Body & Heck, 1973). Chambers did not have frustra or raincaps in 1989, but they were added for the 1992 season. The incorporation of frustra has been shown to reduce ambient air incursions into the chambers and to increase uniformity of ozone concentrations within the chamber (Davis & Rogers, 1980). The raincaps helped us to avoid potential damage from high winds and rain. The exposure system was operative for 95% of the time in 1989 (1731 h out of a total of 1824), and 93% of the time in 1992 (3139 h out of a total of 3360).

At the conclusion of each exposure season, all plants were harvested for biomass determinations. Plants were divided into leaves, stems and roots. Leaf area was measured with a Li-Cor 3000 area meter (Li-Cor, Inc., Lincoln, NE) calibrated against a U.S. National Bureau of Standards certified traceable disk. The total number of leaves on the main stem was counted, and all biomass fractions were dried at 55 °C to constant weight, and weighed to the nearest 0.01 g. In 1992, at the time of harvest the area and weight of the youngest fully formed leaf and of the fourth leaf down from that leaf were measured to see if there were any effects of ozone on leaf size. These leaves were picked because their entire development occurred during the exposures in the chambers.

Data were quality checked and assured using protocols developed at the U.S. Environmental Protection Agency. Precision and accuracy checks were made on all instruments, both before and after

**Table 1.** Seasonal ozone exposures (SUM00, SUM06, and AOT40) for each treatment in 1989 and 1992

Target treatment	SUM00 (ppm h)		SUM06 (ppm h)		AOT40 (ppm h)		Percent of 1.0 × treatment attained†	
	1989	1992	1989	1992	1989	1992	1989	1992
	CF	15.3	9.6	0.0	0.0	0.03	0.00	39
0.5 ×	—*	31.3	—*	0.0	—*	0.00	—*	50
1.0 ×	39.2	63.1	1.9	0.9	1.62	0.78	100	100
1.5 ×	57.1	100.1	17.1	19.4	12.99	15.14	146	159
2.0 ×	77.5	137.0	40.6	53.7	28.30	40.42	198	217

\* Treatment not used in 1989.

† Based on SUM00 values.

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.

#### Statistical analyses

Biomass and growth data were subjected to analysis of variance (ANOVA) to test for ozone treatment effects using SAS (SAS Institute Inc., 1989). The ANOVA model included terms for ozone, chamber with ozone, and plants within chamber. The main effect of ozone was tested using the mean square error for chamber within ozone. The covariate d<sup>2</sup>h was used to reduce variation of tissue biomass data in 1989, but for the 1992 data no covariate was significant. Covariates were not used in either year for leaf area or leaf count data. Residual analysis of tissue biomass data indicated the need for a log transformation to stabilize variances across treatments. ANOVA was also used to test for chamber effects between the open plots and 1.0 × ambient chamber treatment.

Post-ANOVA analyses included orthogonal polynomial contrasts to test for linear and quadratic treatment effects, and Bonferroni's test for comparing the control data with other treatment levels. If the *P* values for treatment effects were near the 0.05 level, then individual and common Weibull exposure-response models with variance components were fitted to combined data to assess the ozone effects on seedling response, and to test for consistency of response across years. The basic Weibull yield-response model was chosen for this analysis because it is flexible and accommodates a range of data patterns (Rawlings & Cure, 1985). The Weibull exposure-response model included fixed effects to account for ozone effects and a random intercept effect to account for environmental or replicate effects. Assuming that the environments encountered in the replicate studies are a sampling from some general population of environments, these extraneous factors are best treated as random effects

for the generalization of observed response curves to the environments of interest (Lesser *et al.* 1990).

The full regression model with variance component for the *i*th siteyear and *j*th chamber within a siteyear can be written as:

$$\log(y_{ij}) = \log\{(A + a_i) \exp[-X_{ij}/B_i]^{C_i}\} + e_{ij}, \quad (1)$$

where  $y_{ij}$  is the chamber mean of tissue biomass,  $X_{ij}$  is the exposure index,  $A$  is the overall mean biomass,  $B_i$  is the exposure level associated with a 63% reduction in yield for the *i*th siteyear,  $C_i$  is the Weibull shape parameter for the *i*th siteyear,  $a_i$  is the random intercept effect, and  $e_{ij}$  is the within-siteyear (error) effect. The fixed parameters are ( $A$ ,  $B_i$ ,  $C_i$ ) and are estimated using the estimated generalized least-squares approach (Gumpertz & Pantula, 1992; Gumpertz & Rawlings, 1992). This mixed effects model is known as the random intercept model (Searle, 1971). Let  $V$  be the covariance matrix of  $y$ , assuming that  $a_i$  and  $e_{ij}$  are independent normal random variables with means zero and unknown variances of  $\sigma_a^2$  and  $\sigma_{ei}^2$ , respectively. Because of heterogeneity of variance across studies, observations were weighted by the inverse of the mean square chamber within ozone from the ANOVA for that data set. The variance components are estimated using the restricted maximum-likelihood approach. The S-PLUS nonlinear mixed-effects program (Lindstrom & Bates, 1988; Lindstrom & Bates, 1990) is used to fit the nonlinear Weibull model with variance components to pooled data.

The reduced model under the null hypothesis of a common response model is:

$$\log(y_{ij}) = \log\{(A + a_i) \exp[-X_{ij}/B]^{C_i}\} + e_{ij} \quad (2)$$

and is compared with the full model using the likelihood ratio test to determine the adequacy of a common model (e.g. Olszyk *et al.*, 1993). Under the null hypothesis of a common model, the likelihood ratio test statistic has an approximate chi-square distribution with two degrees of freedom.

There are several different ozone exposure indices that can be used in response modelling (Lee *et al.*,

1988). We evaluated three such indices, the SUM00, SUM06, and AOT40, for use in weighted nonlinear regression with variance components. Best fits were obtained with the SUM06 and AOT40. We compare the statistical fits for the two exposure indices based on: (1) the *P*-value from the likelihood ratio test of the adequacy of the common model; (2) the estimated variance within siteyears (which is analogous to the mean square error in ordinary least squares regression); and (3) the likelihood ratio value.

## RESULTS

### Ozone exposure results

Ozone treatments in both years, as assessed by the SUM00 index, were substantially different from each other (Table 1). Less separation was apparent for the SUM06 and AOT40 indices, with the two highest treatments showing greater values than the lower treatments for the longer growing season of 1992, whereas the SUM00 for the 1.0 × treatment was correspondingly higher (63.1 ppm h in 1992 compared with 39.2 ppm h in 1989). The seasonal mean concentration was higher in 1989 (21.5 ppb in 1989 vs. 18.8 ppb in 1992). In 1989, SUM06 values ranged from 0 to 40.6 ppm h. In 1992, SUM06 values ranged from 0 to 53.7 ppm h. The frequency of hours above 60 ppb was greater in 1989 than 1992.

SUM00 values in the CF chambers in 1989 were 61 % less than those in the 1.0 × chambers, and 69 % less than the open ambient plots. In 1992, they averaged 85 and 86 % less, respectively. SUM00 exposures in the 2.0 × treatment averaged 1.97 × those in the 1.0 × treatment in 1989, but the 1.0 × and 2.0 × treatments averaged only 0.84 × and 1.66 × that of the open ambient. In 1992, these values were 2.15 ×, 0.92 × and 1.97 ×, respectively.

Ambient ozone values reached or exceeded 60 ppb for 55 h (3 % of the time) in 1989, but for only 20 h (< 1 % of the time) in 1992 (Table 2). The National Ambient Air Quality Standard of 120 ppb was not exceeded in either year. In 1989, the 2.0 × treatment

had a total of 240 h above 60 ppb, and 38 h above 120 ppb, whereas in 1992, it exceeded these values for 401 and 32 h, respectively (Table 2).

### Biomass effects

*Open plots vs. 1.0 × ambient chamber treatment.* No significant chamber effects were found, except in 1992, where height growth was approx. 20 cm less in the open plots than in the 1.0 × treatment (Table 3).

*Treatment effects.* Table 3 shows the results of the variance ratio test for ozone-treatment effects on the log-transformed tissue biomass data. The means in Table 3 have been back-transformed to obtain the original units, and are therefore geometric means and geometric standard errors. The coefficient of variation (equal to the geometric standard error less one) indicates that the experimental variation was an order of magnitude larger in 1992 than in 1989. This is probably because the seedlings were smaller in 1992 at the time they were selected for treatment, had a longer growing season, and genetic variation in growth rate among seedlings was not apparent until later in the season.

The ANOVA results showed highly significant effects of treatment for all biomass fractions in 1989, and less significant, but larger, effects in 1992. Results were similar regardless of whether the SUM06 or AOT40 index was used. Although the trends were similar in both years, the large experimental variation in 1992 substantially reduced the power of the ANOVA to test significant treatment effects. Bonferroni's test was used to make pairwise comparisons between the CF and other treatments, and was considered significant if the Bonferroni values were less than 0.05/*n*, where *n* is the number of treatment means compared with the CF mean (*n* = 3 in 1989, and 4 in 1992). For both data sets, Bonferroni's test showed significant decreases between the CF and 2.0 × treatments for all biomass fractions except the stem (Table 3). Diameters in both years were also smaller in the 2.0 × treatment, although only significantly so in 1989. Neither height nor root:shoot ratio, which includes leaf tissue, were affected by ozone in either year. The leaf area ratio, calculated as the amount of leaf area per total plant dry weight, declined significantly in 1989, but not in 1992.

Leaf count was significantly reduced in 1989 from 73 in the CF treatment to 32 in the 2.0 × treatment (a 56 % reduction), whereas in 1992 it was reduced from 80 to 30 (a 63 % reduction, see Table 3). The loss of leaves resulted in similar magnitudes of leaf area loss (55 % in 1989) but in 1992, because of the greater variability, the effect was marginally significant at the 0.05 level (61 % loss in 1992, see Table 3). There were no effects of ozone on area, weight, or specific leaf mass of the youngest fully expanded leaf

**Table 2.** Number of hours (in 20 ppb classes) above 60 ppb in 1989 and 1992 for open plots and 2.0 × ambient treatment

Ozone class (ppb)	Treatment			
	Open plot*		2.0 × ambient	
	1989 (h)	1992 (h)	1989 (h)	1992 (h)
60–79	55	20	240	401
80–99	0	0	148	194
100–119	0	0	62	84
≥ 120	0	0	38	32

\* Non-chambered ambient values.

**Table 3.** Biomass response (geometric mean  $\pm$  geometric SE) of black cherry seedlings exposed to different ozone treatments in 1989 and 1992. Biomass as dry weight

Parameter	1989										1992									
	Treatment					P values					Treatment					P values				
	CF	0.5 $\times$	1.0 $\times$	1.5 $\times$	2.0 $\times$	AA	AA vs. 1.0 $\times$	Chambers only	CF	0.5 $\times$	1.0 $\times$	1.5 $\times$	2.0 $\times$	AA	AA vs. 1.0 $\times$	Chambers only				
Total (g)	42.54 (1.06)	—	54.31 (1.07)	35.07 (1.06)	29.04* (1.05)	49.19 (1.06)	0.5546	0.0053	44.60 (1.23)	30.93 (1.23)	35.47 (1.23)	28.35 (1.23)	16.77* (1.23)	30.97 (1.21)	0.6249	0.0668				
Leaf (g)	10.04 (1.15)	—	13.73 (1.17)	6.62 (1.15)	4.99* (1.12)	11.17 (1.12)	0.4863	0.0168	13.08 (1.21)	9.52 (1.21)	9.88 (1.21)	8.67 (1.21)	5.04* (1.21)	8.40 (1.19)	0.5328	0.0526				
Shoot (g)	12.47 (1.07)	—	15.05 (1.08)	11.44 (1.07)	10.20 (1.05)	14.77 (1.06)	0.9262	0.0473	10.83 (1.30)	6.12 (1.31)	8.01 (1.30)	7.27 (1.30)	4.60 (1.30)	5.96 (1.29)	0.4302	0.2948				
Root (g)	18.73 (1.06)	—	24.11 (1.06)	15.88 (1.06)	12.12* (1.05)	21.50 (1.04)	0.2343	0.0028	19.69 (1.22)	14.73 (1.23)	16.56 (1.22)	12.00 (1.22)	6.60* (1.22)	15.97 (1.20)	0.8916	0.0270				
Shoot + root (g)	32.16 (1.05)	—	40.72 (1.06)	28.08 (1.05)	23.36* (1.04)	37.80 (1.05)	0.6367	0.0053	31.35 (1.24)	21.27 (1.25)	25.43 (1.24)	19.60 (1.24)	11.60* (1.24)	22.49 (1.22)	0.6688	0.0738				
Root:shoot ratio	0.89 (0.07)	—	0.88 (0.08)	0.93 (0.07)	0.83 (0.05)	0.90 (0.05)	0.9321	0.7351	0.87 (0.08)	0.98 (0.08)	0.98 (0.08)	0.77 (0.08)	0.73 (0.08)	1.18 (0.09)	0.1464	0.1718				
Leaf area (cm <sup>2</sup> )	1574 (1.16)	—	1996 (1.18)	1074 (1.16)	711* (1.12)	1785 (1.11)	0.7392	0.0172	1839 (1.22)	1297 (1.23)	1391 (1.22)	1173 (1.22)	711* (1.22)	1038 (1.20)	0.2924	0.0731				
Leaf area ratio (cm <sup>2</sup> g <sup>-1</sup> )	38.16 (1.99)	—	36.67 (2.18)	33.09 (1.95)	27.55* (1.55)	37.62 (1.34)	0.7334	0.0367	41.72 (2.09)	42.35 (2.13)	40.31 (2.09)	41.77 (2.09)	43.18 (2.09)	37.05 (2.17)	0.3170	0.9022				
Leaf count	72.55 (1.19)	—	85.94 (1.21)	48.13 (1.18)	32.19* (1.14)	70.80 (1.13)	0.3793	0.0312	80.15 (1.20)	56.42 (1.21)	61.37 (1.20)	46.94 (1.20)	29.75* (1.20)	47.02 (1.19)	0.3101	0.0361				
Height (cm)	77.22 (2.29)	—	84.35 (2.51)	78.02 (2.25)	78.32 (1.78)	91.72 (3.17)	0.1061	0.3257	69.97 (6.34)	55.98 (6.47)	69.05 (6.34)	64.90 (6.34)	54.82 (6.34)	48.48 (5.71)	0.0279	0.3469				
Diameter (mm)	9.1 (0.2)	—	10.1 (0.2)	9.2 (0.2)	8.1* (0.2)	10.0 (0.2)	0.9248	0.0049	8.2 (0.6)	6.3 (0.7)	7.3 (0.6)	6.3 (0.6)	5.5* (0.6)	6.2 (0.6)	0.2094	0.1093				

Means marked with an \* are significantly different ( $P < 0.05/n$ , where  $n$  = no. of treatments compared with CF) from the CF treatment mean according to Bonferroni's Test.  $n = 3$  in 1989 and 4 in 1992. Note:  $P$  values are from ANOVA test for main effect of ozone. d<sup>2</sup>h used as a covariate in 1989; no covariate used in 1992. AA = open plots.

**Table 4.** Random intercept models for individual and combined black cherry studies relating chamber mean tissue biomass and the SUM06 exposure index. All biomass variables were transformed by the natural log to stabilize variances within a study

Individual Weibull models								
Biomass variable	Fixed effects model parameters					Random effects variances		
	A	B-1989	B-1992	C-1989	C-1992	Intercept	Error	-LR
Total (g)	43.37	85.3	60.3	1.000	1.000	29.43	0.1688	16.0
Leaf (g)	11.08	49.6	69.5	1.000	1.000	0.00	0.1157	10.6
Shoot (g)	11.00	148.4	76.5	1.000	1.315	12.66	0.1352	15.0
Root (g)	19.74	75.5	50.5	1.000	1.000	4.17	0.1676	15.7
Shoot + root (g)	31.62	97.4	60.2	1.000	1.000	35.29	0.1754	17.3
Aboveground (g)	22.87	93.8	70.4	1.000	1.000	9.54	0.1471	14.4

Common Weibull models								
Biomass variable	Fixed effects model parameters			Random effects variances			LR test	
	A	B	C	Intercept	Error	-LR	$\chi^2$	Prob.
Total (g)	42.19	81.1	1.000	53.46	0.1627	17.3	2.66	0.103
Leaf (g)	10.97	56.6	1.000	0.00	0.1199	11.6	1.96	0.162
Shoot (g)	10.81	130.6	1.054	15.06	0.1316	15.5	1.08	0.584
Root (g)	18.91	69.4	1.067	10.26	0.1726	17.5	3.62	0.054
Shoot + root (g)	30.53	91.3	1.014	58.60	0.1810	18.8	2.97	0.084
Aboveground (g)	22.47	88.9	1.000	14.19	0.1405	15.4	1.88	0.170

Definitions: LR = Likelihood Ratio; LR test evaluates for adequacy of a common model, and has a chi-square distribution under the null hypothesis; due to unequal variances between years, a weighting function was used to fit a common model when combining the two replicate years. The weighting function was calculated as the inverse of the MS chamber within treatment term from the ANOVA; Shoot is any aboveground woody biomass; Aboveground includes leaf plus shoot biomass.

or the fourth leaf down (data not shown). Ozone-induced stipple was noted only in the two highest treatments and will be discussed more fully in a following paper (Neufeld *et al.*, unpublished).

**Exposure-response curves.** Weibull models with variance components were fitted to tissue biomass data in order to examine the relationship between biomass response and ozone exposure for each set of seedlings. Tables 4 and 5 present the full and reduced Weibull models and variance components, where weighted chamber means adjusted for pretreatment height are regressed on the SUM06 and AOT40 indices, respectively. Common models can be fitted for all parameters, as shown by the results of the likelihood ratio test. Because of smaller variations in the 1989 data, the common models are closer to the 1989 regression equations than to the 1992 equations (Fig. 1). The SUM06 and AOT40 indices are better fits to tissue biomass than is the SUM00 index, based on smaller error variances and higher likelihood values. However, the variances of the random intercept are generally larger in fits of biomass to the SUM06 and AOT40 than in fits to the SUM00.

The parameters most affected by ozone in 1989, as estimated from the regressions, were leaf count, area and weight, all of which were highly correlated. Reductions of *c.* 29 and 56% in leaf weight were

found in the 1.5 × and 2.0 × treatments, respectively. Root weight was the second most sensitive parameter, with 20 and 42% losses, followed by total biomass, which was reduced by 18 and 38% in these two treatments. Stem weight, which was reduced only in the 2.0 × treatment, dropped by 24% (Table 4).

In 1992, root weight was more severely affected by ozone, with 32 and 65% losses in the 1.5 × and 2.0 × treatments, respectively. Percent leaf losses were similar to those experienced in 1989, whereas stem losses were the second most severely affected, with 28 and 60% losses. This contrasts with the lower sensitivity of stems in the 1989 seedlings. Total biomass showed larger percentage losses in 1992 than 1989, with 27 and 59% reductions in the 1.5 × and 2.0 × treatments, respectively (Table 4). In both years, losses in the 1.0 × treatment averaged less than 4% for all biomass parameters.

The combined models showed similar patterns to the individual models, with most loss estimates between those for the 1989 and 1992 data sets, with the exception of leaf parameters, for which the common models estimated higher losses (*c.* 61% at 2.0 × treatment). Overall, the combined models show leaf parameters as the most severely affected, followed by roots. Together, these losses amounted to nearly a 48% reduction in total biomass over one

**Table 5.** Random intercept models for individual and combined black cherry studies relating chamber mean tissue biomass and the AOT40 exposure index. All biomass variables were transformed by the natural log to stabilize variances within a study

Biomass variable	Individual Weibull models							
	Fixed effects model parameters					Random effects variances		
	A	B-1989	B-1992	C-1989	C-1992	Intercept	Error	-LR
Total (g)	43.53	59.4	45.5	1.000	1.000	30.98	0.1697	16.1
Leaf (g)	11.13	34.7	51.4	1.000	1.044	0.00	0.1221	10.7
Shoot (g)	11.02	100.2	57.7	1.033	1.298	12.70	0.1442	15.1
Root (g)	19.79	49.8	37.7	1.113	1.000	3.74	0.1779	15.7
Shoot + root (g)	31.72	65.7	45.1	1.044	1.000	35.24	0.1866	17.4
Aboveground (g)	22.93	65.3	53.2	1.000	1.000	10.04	0.1477	14.5

Biomass variable	Common Weibull models							
	Fixed effects model parameters			Random effects variances			LR test	
	A	B	C	Intercept	Error	-LR	$\chi^2$	Prob.
Total (g)	42.61	56.9	1.000	49.06	0.1624	17.2	2.28	0.131
Leaf (g)	10.97	41.3	1.000	0.00	0.1251	12.1	2.86	0.091
Shoot (g)	10.84	85.9	1.123	14.60	0.1321	15.6	0.84	0.656
Root (g)	19.07	47.3	1.142	8.68	0.1713	17.2	3.01	0.221
Shoot + root (g)	30.71	60.9	1.088	54.35	0.1808	18.7	2.62	0.209
Aboveground (g)	22.66	62.6	1.000	13.02	0.1407	15.3	1.58	0.269

Definitions: LR = Likelihood Ratio; LR test evaluates for adequacy of a common model, and has a chi-square distribution under the null hypothesis; due to unequal variances between years, a weighting function was used to fit a common model when combining the two replicate years. The weighting function was calculated as the inverse of the MS chamber within treatment term from the ANOVA; Shoot is any aboveground woody biomass; Aboveground includes leaf plus shoot biomass.

season of growth in the 2.0 × treatment. Losses in the 1.0 × treatment were in the range of 1–2% for all parameters.

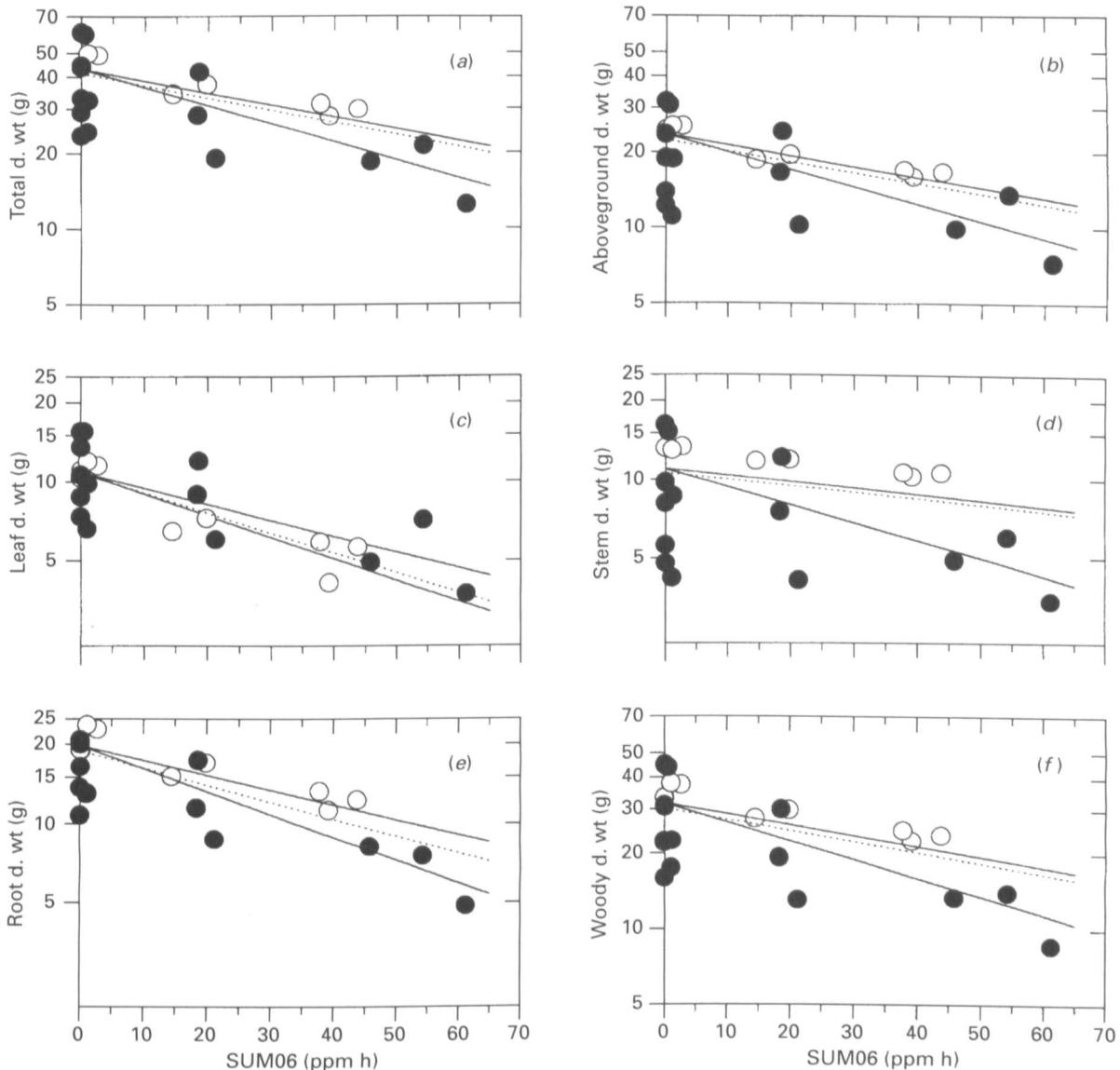
#### DISCUSSION

This is the first study to develop ozone exposure–response relationships for black cherry seedlings in open-top chambers. Samuelson (1994) also studied the responses of black cherry seedlings to ozone in open-top chambers, but found no significant growth effects, or trends, up to 2.0 × ambient. Small sample sizes (three trees per chamber) and a limited number of treatments (three) probably contributed to an inability to detect significant differences.

Earlier studies in CSTRs (Davis & Skelly, 1992), open-top exclusion chambers or outdoor plantations, did not examine the influence of elevated ozone on black cherry (McClenahan & Dochinger, 1981; Long & Davis, 1991; Bennett *et al.*, 1992; Simini *et al.*, 1992), thereby limiting the potential for regression analyses to detect growth responses. The distribution of exposure values obtained in this study, with ozone concentrations ranging from CF to 2.0 × ambient, allowed us to develop exposure–response models for the various biomass fractions, and to compare the results of two separate seasons of exposure on different sets of seedlings.

The only difference between the open plots and 1.0 × ambient chamber treatment occurred in 1992, where height growth in the open plots was less than that in the 1.0 × chambers. Open-top chambers are known to be warmer, less humid, have reduced solar radiation, and different wind conditions, compared with open plots (Unsworth, 1986). For some plants, these changes can affect growth when compared with plants grown in the open (Sanders, Clark & Colls, 1991). In this study, height growth of the 1992 seedlings was reduced in the open plots, probably because of buffeting by the wind (Kozlowski, Kramer & Pallardy, 1991), and perhaps because of etiolation in the reduced light of the chambers. However, biomass, leaf area and leaf count differed very little between these two treatments. We feel confident that the exposure–response relationships developed in this study were not compromised by chamber effects.

Most of the reduction in dry weight caused by ozone occurred because of the loss of leaf material on the main stem and because of reduced root growth. Stem biomass was less sensitive to ozone, a pattern also found by others (Heagle & Camberato, 1987; Cooley & Manning, 1988; Matyssek *et al.*, 1993; Karnosky *et al.*, unpublished). Ozone most likely reduces root growth because: (1) photosynthate is retained in leaves, rather than translocated to roots



**Figure 1.** Exposure response curves for (a) total, (b) aboveground, (c) leaf, (d) stem, (e) root, and (f) woody dry weights, as a function of SUM06 index for black cherry seedlings from 1989 and 1992 studies. Data points are chamber means. ○, 1989; ●, 1992. Fitted lines use the Weibull model, and are predicted responses using random intercepts averaged across data sets for both years. See Table 4 for parameter estimates. Note that all dependent axes are shown with log scaling. (—) individual year models, (····) common model.

(McCool & Menge, 1983; Pell *et al.*, 1994); (2) there is an apparent lower priority by roots for photosynthate, especially under conditions of carbohydrate limitation (Laurence *et al.*, 1994); and (3) most of the photosynthate for roots comes from leaves lower down on the stem (Dickson, 1986), which are the ones that most often show the greatest depressions in photosynthetic rates, owing to ageing and longer ozone exposure (Reich & Amundson, 1985; Pell *et al.*, 1994).

The ratio of leaf area to dry weight, or leaf area ratio (LAR), was significantly lowered by exposure to ozone in 1989, but not in 1992 (Table 3). We suspect that the late initiation of the ozone treatments in 1989, together with the larger size of those seedlings at that time, might have resulted in an

imbalance between root and shoot growth, resulting in a significant, although temporary, reduction in LAR. Conversely, in 1992, the earlier exposures, and the smaller seedlings, might have allowed these plants more time to achieve a balance between root and shoot growth, thus negating any changes in LAR. Of course, the greater variation in 1992 also contributed to the inability to detect changes in LAR, some of which might have been under genetic control.

Matyssek *et al.* (1993) found reductions in LAR for hybrid poplar (*Populus × euramericana*) exposed to ozone. LAR is a relative measure of the carbon assimilatory potential of the whole plant, that is, a surrogate measure of the ratio of carbon assimilation to respiration (West, Briggs & Kidd, 1920), and for

many species is an excellent predictor of growth rate (Poorter & Remkes, 1990). Greater respiratory demand, especially by non-photosynthetic tissues, might lead to reduced carbon availability for certain plants parts and to less growth, until the plant can restore the balance between leaf area and biomass typical of nonstress conditions. A better indicator of future productivity might be the coefficient of leaf area partitioning (LAP), the daily change in LAR (Potter & Jones, 1977). The LAP provides information on both leaf area and weight changes, and is significantly correlated with growth in a wide variety of plants (Potter & Jones, 1977). Although appropriate data are not available in this study to calculate LAP as affected by ozone, extensive knowledge is available on individual rates of leaf photosynthesis in black cherry (Horsley & Gottschalk, 1993), and future experiments should combine these data to model the effects of leaf area loss on growth. This is particularly important for species which have a continuous flushing strategy, whereby newly produced leaves may partly compensate for earlier losses of older leaves, as found for ozone-stressed aspen seedlings (Pell *et al.*, 1994).

Despite the ability to fit both exposure years with a common model, the Weibull parameters from the individual models reveal different patterns of response to ozone between the 1989 and 1992 seedlings. Shafer *et al.* (1993) suggest that the 'B' parameter from the Weibull, which indicates the ozone value required to reduce a response by 63%, is a good indicator of the sensitivity of that response to ozone, even if the predicted 'B' value exceeds the range of the SUM06 values used in the experiment. Following their suggestion, leaf weight was more sensitive in 1989 than 1992, whereas for roots, the sensitivities were reversed. Both stem and total biomass were more sensitive in 1992 than 1989. Overall, with the exception of leaf parameters, 1992 seedlings showed greater sensitivity to ozone than did the 1989 seedlings. This seems to suggest a greater sensitivity in 1992 than 1989, but differences in initial size, growing season length, ozone exposure and timing, might all have contributed to the differences (Pell *et al.*, 1994).

The combined models show that leaf and root biomass are the two most sensitive biomass fractions to ozone, whereas stem biomass is less sensitive. Similar trends have been found in other tree species (Hogsett *et al.*, 1985; Shafer, Heagle & Camberato, 1987; Cooley & Manning, 1988; Matyssek *et al.*, 1993; Karnosky *et al.*, unpublished). In addition, the common Weibull models show average reductions of biomass parameters of the order of only 1–2% from the CF to the 1.0 × treatment. These results compare favourably to those of Simini *et al.* (1992), who detected no significant growth alterations at ambient ozone concentrations, even though they did find that foliar injury was correlated with ozone exposure.

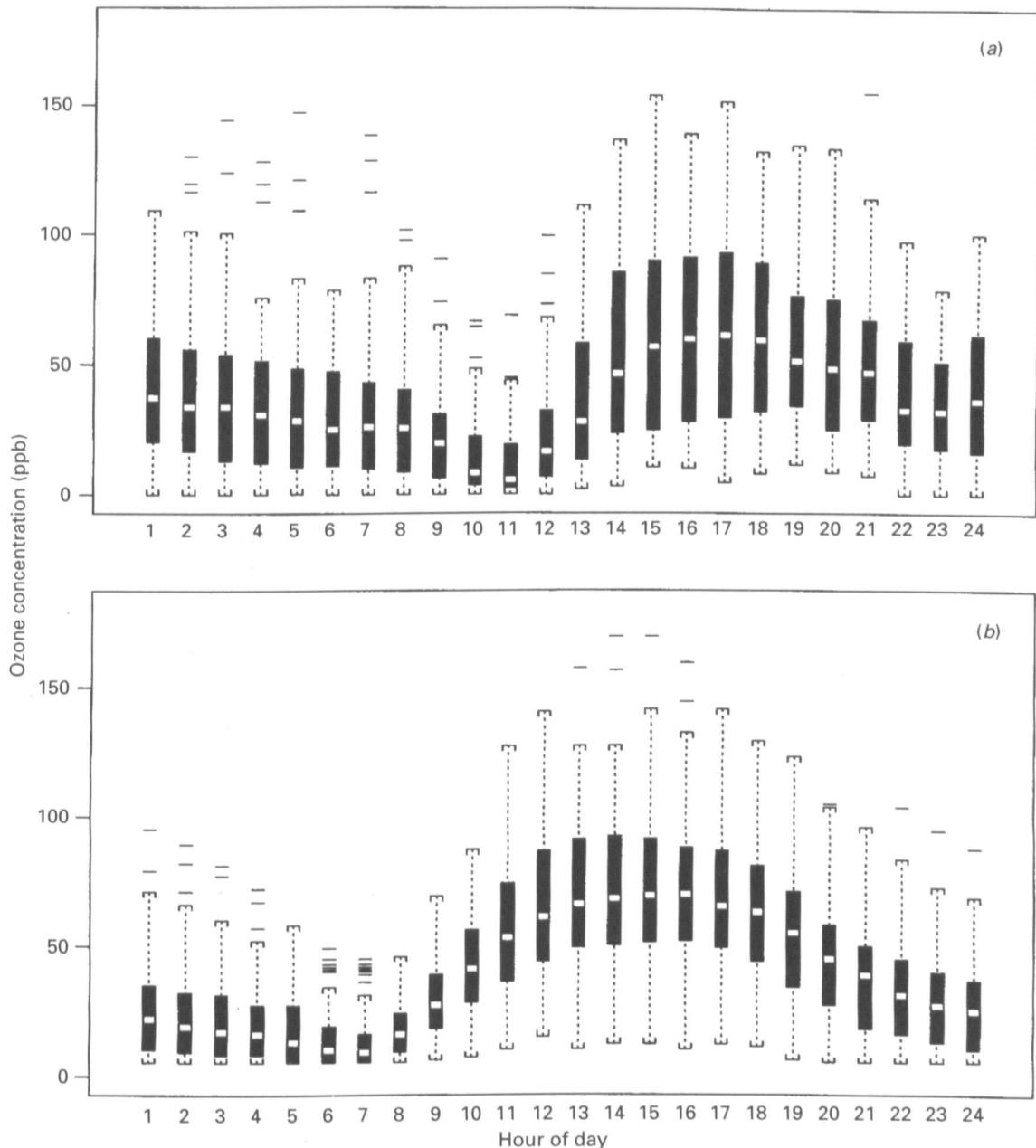
Although ambient concentrations of ozone might cause at most only minor annual growth reductions in black cherry, over the potentially long lifespan of the tree, these might result in large cumulative reductions in growth. Assuming growth losses accumulate in a compound manner, Hogsett *et al.* (1995) showed that a 2% annual growth decline for aspen would result in a 23% growth loss over a 70-yr period.

Such predictions, of course, assume that mature trees react like seedlings, and potted seedlings like trees in the field. Recent investigations with red oak (*Quercus rubra* L.) and giant sequoia (*Sequoiadendron giganteum* Bucholz) have shown significant differences between seedling and mature tree responses to ozone (Samuelson & Edwards, 1993; Grulke & Miller, 1994). Currently, work on foliar ozone effects in mature black cherry trees is being conducted in Great Smoky Mountains and Shenandoah National Parks (Chappelka *et al.*, 1992; Chappelka, Renfro & Somers, 1994; Samuelson, pers. comm.). Data from these studies are a first step toward comparing responses of seedling and mature trees to ozone.

The 1.5 × and 2.0 × treatments have similar ozone dynamics as the Piedmont region of North Carolina during years with large ozone pollution. Figure 2 is a box plot of our 2.0 × treatment in 1989, together with ambient ozone data for a rural site in Mecklenberg County near Charlotte, NC in 1988; both the pattern and magnitude are quite similar. From this study, we suggest that black cherry seedling growth in the Piedmont has the potential to be adversely affected during years of high ozone exposure. Such a prediction assumes, among other things, site conditions conducive to ozone uptake (adequate soil water and nutrients, appropriate temperatures and humidities, etc.), similar sensitivity of field grown plants as compared with potted seedlings, and similar genetic tolerance to ozone for genotypes from these two regions.

SUM00 values for the high-elevation monitoring sites in GRSM are similar in magnitude to the 1.5 × and 2.0 × treatment SUM00 values in our exposure study, although with quite different dynamics (Neufeld *et al.*, 1992). Given that black cherry grows at these elevations, and that significant growth reductions were found in the 1.5 × and 2.0 × treatments, we suggest, cautiously, that black cherry seedlings growing at high altitudes in GRSM might also be at risk from ambient ozone, notwithstanding the above assumptions. Because of the large elevational gradient, ecotypic differences might exist between low and high altitude black cherry that should be evaluated for their effects on ozone response.

Despite the large difference in initial size of seedlings between the two exposure years, the length of the exposure seasons, and the different exposure values, the responses of the two seedling sets were



**Figure 2.** Diurnal patterns of hourly ozone concentrations for (a) the 2.0 × ambient treatment in Great Smoky Mountains National Park over the exposure period in 1989 and (b) a rural ozone monitoring site in Mecklenberg County, North Carolina over the exposure season from May to October, 1988. Data from the U.S. Environmental Protection Agency's Aerometric Information Retrieval System database (AIRS ID = 371191009; latitude = 35° 35' N and longitude = 80° 69' E). Key: white bar in centre = median; black bar = range between 25th and 75th percentiles; dotted lines = 2 × the midrange (inner and outer hinges, approx. 3 standard deviations, or 99% of the data points); horizontal bars = extreme values (each bar is one event).

remarkably similar. Common exposure models were developed for all biomass fractions. This suggests that one season of exposure was minimally adequate for describing the overall response to ozone, despite the aforementioned differences in responses between years. Certainly more multi-year studies are warranted before making broad statements regarding sensitivities of species to ozone. Failure to repeat studies in a chronic problem in ecology, and even highly controlled experiments are sometimes not reproducible (Primack & ShiLi, 1991). Shafer *et al.* (1993) found that rankings of ozone sensitivity

among 12 loblolly pine (*Pinus taeda* L.) genotypes could change from one year to the next, but only primarily in those families with intermediate sensitivities. Those with extreme responses were ranked consistently from one year to the next.

Finally, the difference in the magnitude of variation between the two data sets warrants some mention. We think the larger variation in the 1992 data set represents the cumulative effects of a longer growing season, larger sample size, and late expression of genetic differences in growth potential. The 1989 seedlings were larger and older when they

were culled for uniformity before being placed in the chambers, hence the much smaller possibility of variation between replicate plants. This raises the question of when seedlings should be selected for inclusion in exposure studies. Certainly the possibility exists that early selection will retain more genetic variation than late selection, leading to wide variances at the end of the experiment. This will make it difficult to separate treatment effects statistically, since many exposure studies have low power owing to small sample sizes (Pye, 1988; Samuelson, 1994). Early selection might require the experimenter to increase the number of treatment replications (i.e. chambers) to account for the anticipated high levels of variation. On the other hand, reducing variations among replicate plants by selecting older individuals that already have established growth patterns, might result in the elimination of extreme genotypes, leading to false conclusions about the extent of ozone tolerance of individuals within the population.

Our purpose in these studies was to estimate population-wide responses without regard to family or site characteristics. The large variation in the 1992 data set suggests the existence of a substantial pool of genetic variation in black cherry, and future studies should concentrate on the genetics of ozone tolerance in this species. In a series of recent papers (Berrang *et al.*, 1986; Berrang, Karnosky & Bennett, 1989, 1991; Shafer *et al.*, 1993), considerable genetic variation in response to ozone for aspen and loblolly pine trees has been found, and Berrang and co-workers have even suggested that ozone-susceptible aspen genotypes are being selected against in the field. Certainly the possibility exists that the same might be true for black cherry seedlings, especially given their extreme sensitivity to elevated ozone, as shown in the present study.

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