

Effect of Ozone Exposure on Seasonal Gas Exchange of Five Western Conifers¹

Nancy E. Grulke,² Paul R. Miller,² Theodor D. Leininger³

Abstract

Five species of western conifers (*Pinus ponderosa*, *Abies concolor*, *Pseudotsuga menziesii*, *Abies lasiocarpa*, and *Picea engelmannii*) were exposed, in two standard open-top exposure chambers per treatment, to charcoal-filtered air and a simulated diurnal ozone exposure profile (120 d sum of 136 ppm-h) to test their relative sensitivity. CO₂ exchange rate (CER), stomatal conductance (gs), and total leaf nitrogen of current year foliage were measured at 1-month intervals. *P. ponderosa* was the most adversely affected by ozone, based on reductions in mid- and late season CER under saturating light (1.18 vs. 2.17 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), lower light compensation point (43 vs. 33 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$), and lower CER at a given gs value. *P. menziesii* showed reduced CER and higher gs by the end of the experimental ozone exposure. *A. concolor* and *P. engelmannii* showed no reduction in CO₂ uptake and no clear effects of ozone exposure on gs. Adverse ozone effects on biomass or growth in the year of exposure were seen for *A. lasiocarpa* and *P. ponderosa*, whereas *A. concolor*, *P. engelmannii* and *P. menziesii* showed adverse effects only in the year after exposure. Few consistent patterns in which plant component was affected were found, either in the year of exposure or the year after exposure. An analysis of the number of chambers required or the difference between treatment means necessary to obtain statistical significance with the population variance measure was an important result.

Introduction

Considerable variations in the response of trees to air pollutants may be caused by differences in seed source, nutritional status of plants and other stressors, plant age, and plant phenological stage (Bytnerowicz and Grulke 1992). Many experimental exposure studies have analyzed the effects of pollutants on gas exchange at one phenological period: near the end of the growing season when cumulative experimental exposure to the pollutant is at a maximum. However, because gas exchange rates may be at a minimum, treatment effects may be difficult to distinguish statistically.

Plant response to ozone may not necessarily be cumulative, but relatively few studies have studied this by examining seasonal responses during ozone exposures (Byres and others 1992, Grulke and others 1989, Hanson and others 1994, Samuelson and others 1996, Weber and others 1993). As long as concentrations are sufficiently low, ozone may have little effect on gas exchange. However, high ozone uptake rates have been found to reduce stomatal conductance (gs) (Weber and others 1993).

This study determined the relative susceptibility of five species of western conifers — *Abies concolor* [Gord. & Glend.] Lindl., *Abies lasiocarpa* [Hook.] Nutt., *Picea engelmannii* Parry, *Pinus ponderosa* Dougl. ex. Laws, and *Pseudotsuga menziesii* Franco — to ozone exposure by analyzing gas exchange characteristics (CO₂ exchange rate, stomatal conductance, and dark respiration), growth, and biomass allocation.

Materials and Methods

Plant Culture

Two-year-old, bare-root *A. concolor* seedlings were obtained from the California Department of Forestry nursery in Ben Lomond (seed zone 531.50). One-year-old, container-grown *P. ponderosa* seedlings were obtained from the same nursery (seed zone 522.20, lot 3245). Two-year-old *A. lasiocarpa* seedlings in containers were obtained from Plants of the Wild, Tekoa, Washington (seed from south of Lewiston, Idaho, 1,372 m). Two-year-old, bare root stock of *P. engelmannii* (seed source Warm Lake, Idaho) was obtained from Lucky Peak Nursery. One-year-old container-grown seedlings of *P. menziesii* were obtained from Rex Timber, Inc., Cottage Grove, Oregon (seed zone 471, 620 m).

¹ An abbreviated version of this paper was presented at the International Symposium on Air Pollution and Climate Change Effects on Forest Ecosystems, February 5-9, 1996, Riverside California.

² Research Plant Physiologist and Research Plant Pathologist, respectively, Pacific Southwest Research Station, USDA Forest Service, 4955 Canyon Crest Drive, Riverside, CA 92507.

³ Research Plant Pathologist, Southern Research Station, USDA Forest Service, Box 227, Stoneville, MS 38776.

Seedlings (6.4 cm dia., 25.4 cm tall) were potted in a mix consisting of equal parts Promix B¹ and perlite. Before fumigation, plants were kept in an activated charcoal-filtered, evaporative-cooled greenhouse adjacent to the fumigation site. The greenhouse and fumigation site were installed at the Kenworthy Ranger Station (33° 37' N, 116° 37' W), in the San Jacinto Mountains, 22 km SE of Idyllwild, and 80 km W of Palm Springs, California. The elevation of the site was 1,387 m. A drip irrigation system was used to water and fertilize the plants (North Carolina State University fertilizer mix). Plants were watered two times weekly to pot capacity. Nutrients were applied every 1-2 weeks through the irrigation system from March through October 11, 1988.

Ozone Exposure

Seedlings were exposed to either charcoal-filtered air (CF), or charcoal-filtered air augmented with ozone (+O₃) under ambient conditions in open-top exposure chambers (Heagle and others 1979). Chambers operated from June 4 (Julian day 155) through October 11, 1988. For the elevated ozone treatment, a 30 d diurnal profile was developed from average air quality characteristics during the summer months in the midwest (1.8 times ozone exposure level; Hogsett and others 1989; Lefohn and others 1986a, 1986b). The 30 d base profile was repeated about four times in the 124 d exposure period (*fig. 1*). The ozone exposure in each treatment was estimated from the following equations:

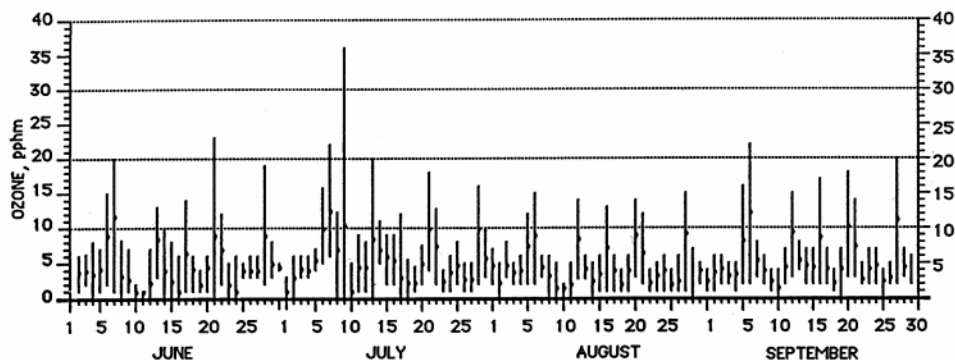
$$\text{CF:} \quad y = 0.278x - 1.44 \quad (1)$$

$$\text{O}_3: \quad y = 1.118x - 1.66 \quad (2)$$

in which y was ozone exposure in ppm-h and x was number of days of exposure.

Ozone was generated, distributed, and sampled within each chamber by using a computer-controlled program with a data acquisition system (Hewlett Packard).⁴ Oxygen (O₂) was supplied to an ozone generator (Griffin Technics, Model GTC-0.5b), and distributed to chambers with mass flow controllers (Datametrics, Type 825). Ozone was sampled from the center of each chamber by using a sequential sampler (Scanivalve, custom-made electronics). Blower boxes provided a chamber exchange rate of 2 min⁻¹ and ozone concentrations were determined by ultraviolet photometry (Dasibi, Model 1003AH, calibrated bi-weekly on site). Fiberglass dust filters and activated charcoal-filters were in place for all chambers. Concentrations of ozone in chambers were recorded hourly as were ambient ozone, chamber air temperature, soil temperature at 5 cm, and total global radiation (Licor 200SZ).

Figure 1 — Seasonal course of daily mean, maxima, and minima ozone concentration in the elevated ozone chambers. The lowest ozone concentrations occurred in the morning from 0700 to 0900, and the highest ozone concentrations occurred between 1500 and 1700 hr.



Gas Exchange

Before experimentation, a typical subset of plants for physiological measurement was placed in each chamber: all had one terminal bud, appeared to be in the same phenological stage, and were visually similar in height, numbers of side branches, and foliage color. Bud flush had occurred before placement in chambers. Three seedlings of each species per chamber and two chambers each for charcoal filtered air and elevated ozone treatments were available for physiological

⁴ Mention of trade names or products is for information only and does not imply endorsement by the U.S. Department of Agriculture.

measurements. Means of each plant were obtained from two to four sequential runs (three to four observations averaged per run). Gas exchange from the three seedlings of each species in one chamber were averaged together to obtain the chamber mean and standard error. The analysis of variance (ANOVA) calculations were strictly applied to the chamber means (one degree of freedom within a treatment).

The conifers were exposed to charcoal-filtered air and elevated ozone in open-top chambers for one growing season. The conifers included *Abies concolor* [Gord. & Glend.] Lindl., *Abies lasiocarpa* [Hook.] Nutt., *Picea engelmannii* Parry, *Pinus ponderosa* Dougl. Ex Laws, and *Pseudotsuga menziesii* Franco. Gas exchange of current year foliage was measured four times during the exposure period from early June through late September. Dark respiration rate and light response of *A. concolor*, *P. ponderosa*, and *P. menziesii* were measured once at the end of the experimental exposure and the growing season. Biomass was measured twice on a separate set of seedlings: at the end of the experiment in the fall, and in the fall after 1 year of "recovery." The seasonal pattern of gas exchange and their relative susceptibility to ozone exposure of the five conifers was measured. For each species, gas exchange of current year foliage from the same three seedlings from each chamber was measured at the beginning of the exposure period in early June and subsequently at 1-month intervals through late September. CO₂ exchange rate (CER) and stomatal conductance (gs) were measured with a Licor 6200 photosynthetic system (Lincoln, Nebraska). A 0.25 L cuvette was used for all species except *P. ponderosa*. For this species, a custom cuvette (12 x 15 x 15 cm) was constructed from Acrylite lined with Teflon film so that shoot architecture was not disturbed during measurement. Leak rates of the custom cuvette were low and comparable to the purchased cuvette.

Gas exchange measurements were made outside fumigation chambers between 0930 and 1530 h. Plants of the same species were out of chambers approximately 1 h before measurement (Hinckley and others 1990) and were measured at the same time of day throughout the growing season. During all measurement sessions, plants from CF chambers were alternated with ozone chambers to nullify possible diurnal effects. In addition to biweekly watering, all plants were watered to capacity 0.5 d before measurements.

Gas exchange was measured within 1 minute. Gas exchange measurements for the ozone experiment were taken at ambient light within a photosynthetic photon flux density (PPFD) range of 750-1525 $\mu\text{mol m}^{-2} \text{s}^{-1}$, leaf temperature of 29-37 °C, and vapor pressure deficits (VPD) of 2.75-4.50 kPa. CO₂ exchange rate and stomatal conductance appeared to have a flat response across this range of physical factors, other than light, for which lower levels could have limited gas exchange up to 10 percent. Dark respiration rate of *A. concolor*, *P. ponderosa*, and *P. menziesii* was measured near the end of the season-long experiment, approximately 0.5 h after sunset. Measurements were taken within an air temperature range of 16-20 °C.

CER was calculated on the basis of needle surface area. Surface area was calculated from simple geometric models of leaf surfaces with consideration for surfaces with stomata. Measures of needle width (in the middle) or fascicle diameter (just above the sheath) and length were made with a digital micrometer.

Light response was determined near the end of the summer ozone exposure period. Four frames consisting of a light source, heat/light separation mirror, and neutral density filters were used to control light intensity (Grulke and others 1989). The same plants measured for seasonal response were also measured in this analysis. Plants were allowed to equilibrate for 0.5 h at each light level before gas exchange was measured. Gas exchange measurements were made between cuvette temperatures of 22-26 °C. For light response determinations of *P. ponderosa*, three fascicles were enclosed in a 0.25 L cuvette (spread to reduce self-shading). A simple leaf model was used to define the relationship: $(\text{CER} = P_{\text{max}} (1 - e^{-\phi I / P_{\text{max}}}))$, in which ϕ is apparent quantum efficiency and I is PPFD; Thornley 1976). The CO₂ light compensation point and CER with saturating light were calculated from the fitted regression equations for each plant.

Leaf Nitrogen

Leaf tissue was sampled for nitrogen content just after the monthly gas exchange measure by using a standard micro-Kjeldahl digest technique on dried tissue (65 °C, 24 h), ground to pass a 40 mesh. Digests were run on an auto-analyzer (Technicon) with standards, blanks, replicates, and duplicate runs every 20 samples. Samples with ≥ 2.5 percent error ($[S.D./x] \times 100$ percent) were re-analyzed on a Carlo-Erba NA1500 Series 2 analyzer.

Biomass and Plant Size

Plant height (mm) and stem diameter (0.01 mm) was measured before experimental exposure for six additional seedlings per chamber. At the end of the experiment, plant height, stem diameter, and biomass allocation to roots, stem, older needles, and new (current year) needles were determined for the same six seedlings. The change in seedling height and stem diameter was relativized by using the initial measures taken before the experiment ($\Delta \text{ht}/\text{ht} \times 100$ percent). For yet another set of six seedlings per chamber, plant size and biomass allocation was measured after 1 full year of growth in a charcoal-filtered air glass house after experimentation.

Results

CO₂ Exchange Rate

Seasonal trends in CER were different for each species: maximum CER occurred at the first sampling date for *P. ponderosa*, at the second sampling date for *Abies lasiocarpa*, and at the last sampling date for *Abies concolor*, *Picea engelmannii*, and *Pseudotsuga menziesii* (fig. 2). The maximum observed CER varied nearly twofold for current year foliage between the species (*A. concolor*, 1.68 ± 0.42 ; *A. lasiocarpa*, 1.42 ± 0.08 ; *P. engelmannii*, 2.40 ± 0.11 ; *P. ponderosa*, 1.52 ± 0.11 ; *P. menziesii*, 2.50 ± 0.22 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Particularly low values of CER were obtained on the second sampling date for *P. menziesii* because of high vapor pressure deficits.

Only *P. ponderosa* showed significant effects of elevated ozone, which were measured at the second sampling date (fig. 2). Despite the few chambers used, several trends are apparent. Although initial CER in the elevated ozone treatment was sometimes greater than that of the charcoal filtered air treatment (e.g., *A. concolor*, *P. engelmannii*), the end of season mean CER was lower in the elevated ozone treatment for all species. After the exposure regime began, the largest differences in CER between the elevated ozone and charcoal filtered air treatments occurred early season for *A. concolor* and *A. lasiocarpa*, and midseason for *P. ponderosa*. We found no differences attributable to treatment for both *P. engelmannii* and *P. menziesii*.

Because of high within-chamber variability due to an uneven number of sensitive and tolerant individuals in each chamber, and because the seasonal gas exchange patterns were different for the plants within each chamber, significant differences were not found between treatments (except for *P. ponderosa*). For example, one *A. concolor* seedling had a positive parabolic pattern, one had a constantly declining seasonal gas exchange pattern, while the remaining four seedlings within a treatment had a negative parabolic pattern of seasonal gas exchange. The net effect of these differing seasonal patterns of gas exchange is a relatively flat seasonal response with high standard errors (see *A. concolor*, fig. 2). Neither condition (sensitivity or asynchronous seasonal patterns of gas exchange) could have been known before the experimental exposure.

Measured at the end of the experiment, dark respiration was higher in seedlings exposed to elevated ozone than charcoal filtered air treatments for all species, but differences were not statistically significant (table 1).

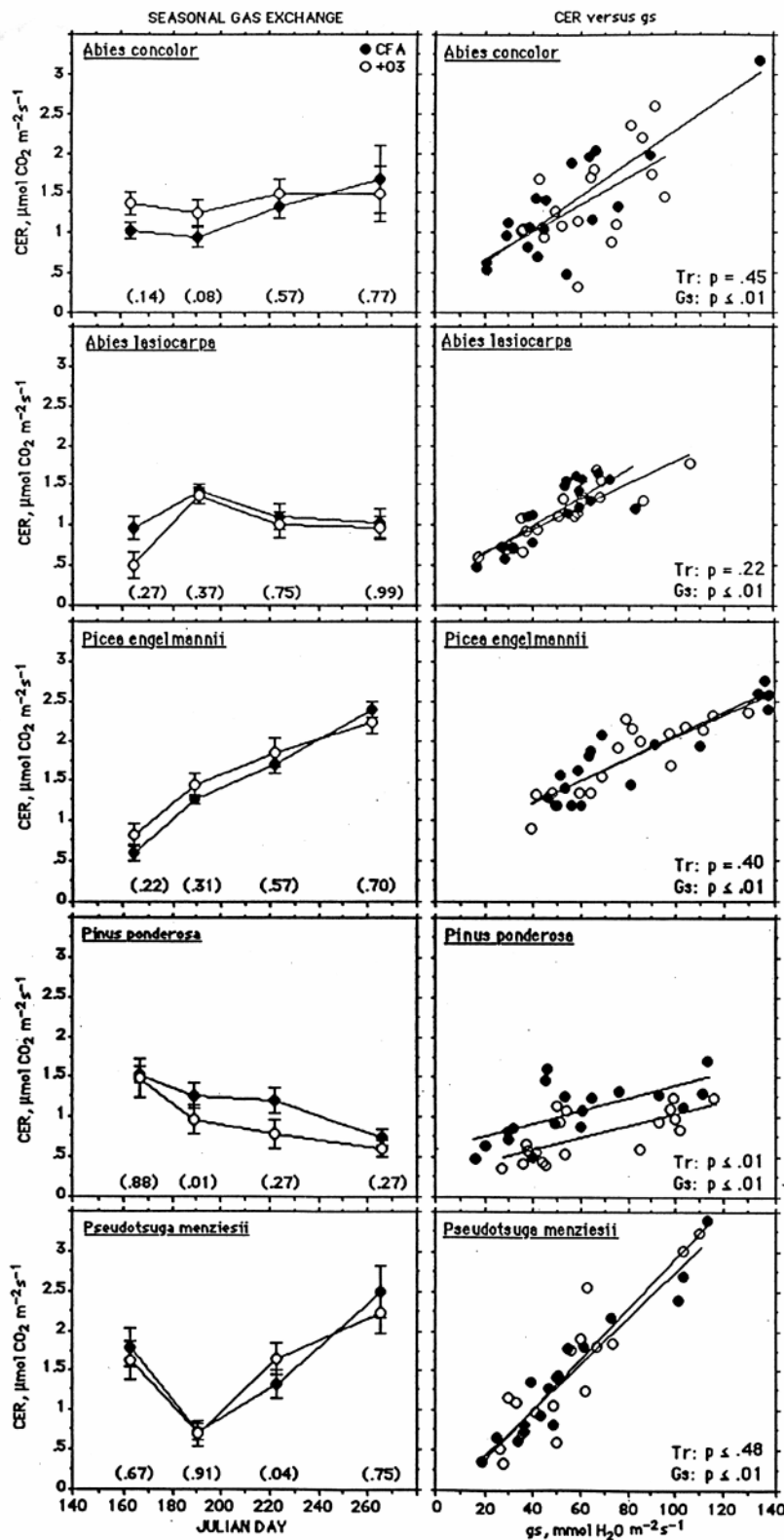


Figure 2 — Seasonal trend in CO_2 exchange rate (CER) for charcoal-filtered (CF) air (●) and elevated ozone treatments (○) for five species of western conifers. Symbols and error bars represent averaged means for the two chambers ± 1 S.E. Values in parentheses are probabilities that the two means are significantly different in a one-way ANOVA. The relationship between CO_2 exchange rate (CER) and stomatal conductance (gs) for the five species for month 2-4 of treatment is also given. Symbols represent average values for 2-4 runs per plant; in all cases, standard error was contained within the symbol. Values in lower right-hand corner are probabilities that the treatment (Tr) and stomatal conductance (gs) were significantly different. Slopes of the lines were not significantly different between treatments for any species.

Leaf Nitrogen

No seasonal trends in needle nitrogen content were apparent for any of the species. Needle nitrogen differed significantly between the charcoal filtered air and elevated ozone treatments averaged over the duration of the experiment for *A. concolor*, but not for the other species (table 2). CER based on leaf nitrogen content did not improve statistical separation of response to experimental treatment (fig. 1).

Stomatal Conductance

Seasonal patterns in stomatal conductance (gs) largely followed CER (fig. 1). However, after a month of treatment exposure, gs was generally greater in elevated ozone treatment for *A. concolor*, *A. lasiocarpa*, and *P. ponderosa* than in the charcoal-filtered air treatment. CER was plotted as a function of gs to further test for the effects of elevated ozone exposure (fig. 1) for months 2 to 4 of treatment exposure. The slopes of the regressions were not significantly different for four of the species (Draper and Smith 1981). For *P. ponderosa*, CER was significantly reduced in the elevated ozone treatment relative to the charcoal filtered air treatment at the same gs, suggesting reduced water use efficiency. The differences in CER were greater than differences in dark respiration (table 1).

Light Response

Light compensation points were not significantly different between treatments for *A. concolor*, *P. ponderosa*, or *P. menziesii* (table 3) using a one-way ANOVA. CER at light saturation (1,600 $\mu\text{mol m}^{-2}\text{s}^{-1}$) was significantly different between treatments for *P. ponderosa*, but not for *A. concolor* or *P. menziesii* (table 3). The apparent quantum efficiency (the slope of the regression line between 0 and 100 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$) for *P. ponderosa* seedlings growth in charcoal-filtered air and elevated ozone was statistically significant ($p\leq0.01$) (Draper and Smith 1981). The difference in light response between treatments in *P. ponderosa* was much greater than the difference in dark respiration observed (table 1).

Biomass Allocation

Biomass allocation to roots, stems, old needles (produced before this experiment), and new needles (current year) was analyzed for seedlings immediately after the growing season, and 1 full year after experimental exposure (fig. 3). Some species

Table 1 — Dark respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at the end of the experiment for charcoal-filtered (CF air) and elevated ozone (+ O₃) treatment for the five conifer species studied.

Species	CF air ¹	+ O ₃ ¹	p ²
<i>Abies concolor</i>	0.21±0.00	0.22±0.02	(0.52)
<i>Abies lasiocarpa</i>	0.16±0.01	0.17±0.02	(0.72)
<i>Picea engelmannii</i>	0.15±0.01	0.16±0.01	(0.14)
<i>Pinus ponderosa</i>	0.20±0.04	0.24±0.03	(0.50)
<i>Pseudotsuga menziesii</i>	0.30±0.03	0.34±0.00	(0.24)

¹ Mean and error represent averaged means for the two chambers and within-chamber standard error in each treatment.

² Probability for significant differences between values (one-way ANOVA).

Table 2 — Leaf nitrogen (percent) averaged for the four sampling points throughout the duration of the experiment for charcoal-filtered (CF air) and elevated ozone (+ O₃) treatment.

Species	CF air ¹	+ O ₃ ¹	p ²
<i>Abies concolor</i>	1.38±0.08	0.98±0.01	(±01)
<i>Abies lasiocarpa</i>	1.27±0.06	1.14±0.04	(0.11)
<i>Picea engelmannii</i>	1.84±0.10	1.90±0.11	(0.72)
<i>Pinus ponderosa</i>	1.20±0.06	1.15±0.05	(0.51)
<i>Pseudotsuga menziesii</i>	1.11±0.08	1.13±0.12	(0.89)

¹ Mean and standard error.

² Probability for significant differences between values (one-way ANOVA).

showed adverse ozone effects on biomass or growth in the year of exposure (*A. lasiocarpa*, *P. ponderosa*), some showed only adverse effects in the year after exposure (*A. concolor*, *P. engelmannii*, *P. menziesii*). There were few consistent patterns in which plant component was affected, either in the year of exposure or the year after exposure. For all species, the percent biomass allocation to older needles was lower for seedlings grown in elevated ozone chambers relative to charcoal-filtered air chambers, but the differences were not significant (table 4). Total biomass was lower for seedlings in elevated ozone than charcoal-filtered chambers immediately after exposure for all species except *P. ponderosa*, where seedlings were larger (+10.6 percent in total biomass, and 10.1 percent in dia.) at the end of the experimental exposure. Plants in the elevated ozone treatments were also larger in May before experimental exposure. Two species also had lower biomass allocation to new needles (*A. lasiocarpa* ($p=0.01$), *P. menziesii*, $p=0.57$), but *A. concolor*, *P. engelmannii*, and *P. ponderosa* had greater allocation to new needles.

Discussion

In this study testing the sensitivity of five western conifers to ozone, *P. ponderosa* appeared to be the most sensitive to ozone on the basis of reductions in CER (in midseason, CER versus gs responses, and in light saturation experiments at the end of the growing season). Among all of the species, *P. ponderosa* had the greatest gs early in the season when young leaf tissues were most likely to be damaged by oxidants. In general, species of pine appear to be more sensitive to ozone exposure than any other conifer (Bytnerowicz and Grulke 1992).

Although *A. lasiocarpa* had a seasonal pattern of gs similar to that of *P. ponderosa*, few symptoms of sensitivity to ozone developed. In this study, as well as in their natural habitat, CER and gs of *A. concolor* (Conard and Radosevich 1981) and *P. engelmannii* (Carter and others 1988, DeLucia and Smith 1987) increased gradually through the growing season. Comparisons of instantaneous gas exchange measures showed that both species appear to be relatively resistant to ozone exposure (fig. 1).

Instantaneous measures of gas exchange as well as biomass of *P. menziesii* did not statistically demonstrate evidence of damage by exposure to ozone. However,

Table 3—Light compensation point and CO₂ exchange rate at the end of the exposure for charcoal-filtered air (CF air) and elevated ozone (+ O₃) treatments.

Species	CF air ¹	+ O ₃ ¹	p ²
Light compensation point ³			
<i>Abies concolor</i>	28 ± 5	28 ± 4	(0.98)
<i>Pinus ponderosa</i>	47 ± 5	49 ± 6	(0.86)
<i>Pseudotsuga menziesii</i>	20 ± 4	19 ± 4	(0.89)
CO ₂ exchange rate at light saturation ⁴			
<i>Abies concolor</i>	3.17 ± 0.34	3.21 ± 0.36	(0.93)
<i>Pinus ponderosa</i>	2.67 ± 0.19	1.21 ± 0.24	(0.001)
<i>Pseudotsuga menziesii</i>	4.84 ± 0.51	5.45 ± 0.39	(0.37)

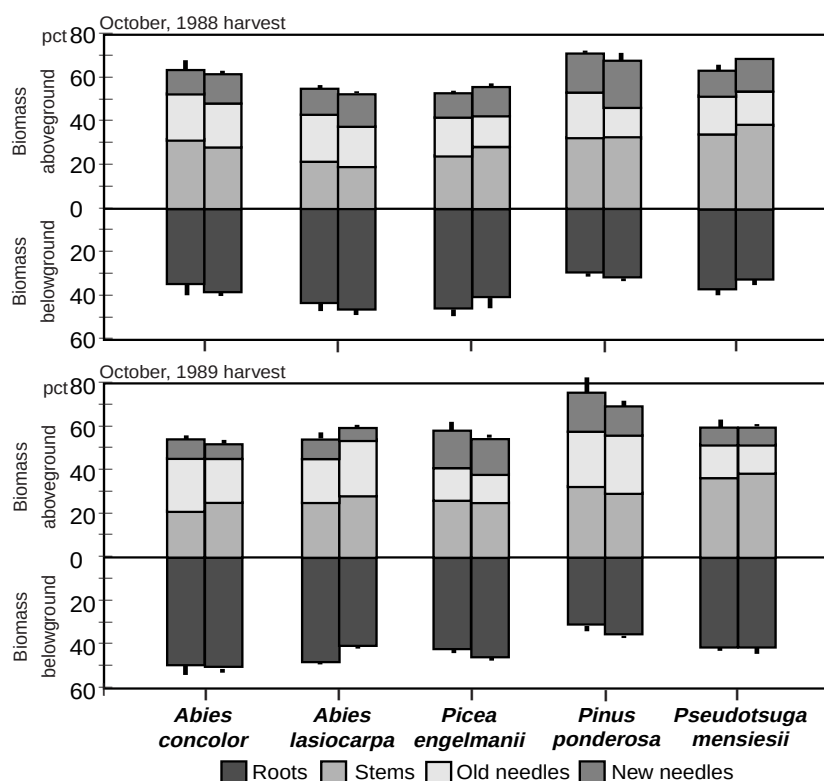
¹ Mean and error represent averaged means for the two chambers and within-chamber standard error in each treatment.

² Probability for significant differences between values (one-way ANOVA).

³ μmol quanta m⁻² s⁻¹.

⁴ μmol CO₂ m⁻² s⁻¹.

Figure 3 — Change of biomass allocation by percent for seedling growth in charcoal-filtered air (first column) compared to those in elevated ozone chambers (second column). Two sets of seedlings were harvested: (a) immediately after the growing season of experimental exposure and (b) after 1 full year of growth in a glass house supplied with charcoal-filtered air. Bar lengths represent the means of the two chambers and ± 1 S.E.



all species showed a trend of increased instantaneous measures of dark respiration, and subsequent decreased biomass of older needles (increased needle turnover rates) immediately after the experiment. Most species (except *P. menziesii*) showed a trend of decreased biomass of new needles 1 full year after experimental exposure. In another study of *P. menziesii* exposed to elevated ozone in Washington, measures of instantaneous gas exchange did not reflect significantly reduced stem diameter and root to shoot biomass at the end of a 2-year study (Hinckley and others 1990).

Instantaneous values are appropriate to understand what plant processes may be sensitive to oxidants, but measuring long-term biomass changes may be a better measure to detect damage. Modeling efforts that incorporate the small differences in gas exchange and subsequent carbon allocation within the plant year after year may demonstrate the consequences of subtle changes in carbon balance more clearly.

Few of the differences observed between the charcoal-filtered air and the elevated ozone treatments were significant. The lack of statistical significance was primarily because of high within-chamber variance. Even if chamber effects were assumed to be null and each plant were treated as a true replicate, there were few additional statistically significant differences between treatment means. In many pollutant exposure studies, plants are tested after the maximum cumulative exposure period, often at the end of a growing season, when gas exchange rates are declining and the ability to statistically distinguish between means is the least powerful. In this study, the statistically significant differences between the charcoal filtered air and elevated ozone treatments occurred in the second month of exposure for *P. ponderosa* (fig.1). Cumulative exposure to ozone was not necessarily correlated to peak deleterious effects on gas exchange in other studies (Byres and others 1992, Weber and others 1993). In this study, high variability in plant response within a chamber and too few chambers significantly hindered the evaluation of the effects of exposure to elevated ozone.

To better clarify the lack of statistical significance, and assuming the same within-chamber standard error, the difference between treatment means required for significance at the $p = 0.05$ level, or the number of chambers of plant gas exchange that would have been required for the means to have significance was calculated (table 5). Untenably large numbers of chambers would

have been required to demonstrate statistical significance between treatments for all species other than *P. ponderosa*.

One source of high population variance may be naturally occurring genotypes that are “sensitive” or “resistant” to pollutants (Ernst and others 1985, Taylor 1978). Evidence for differences in population sensitivity to ozone was found in different individuals of *P. jeffreyi* (Patterson and Rundel 1989), different seed sources of

Table 4—Magnitude and direction of change of biomass allocation by percent for seedlings grown in charcoal filtered air compared to those in elevated ozone chambers. Two sets of seedlings were harvested: (A) immediately after the growing season of experimental exposure and (B) after 1 full year of growth in a glasshouse supplied with charcoal-filtered air.

A. 1988 October Harvest

	<i>Abies concolor</i>	<i>Abies lasiocarpa</i>	<i>Picea engelmannii</i>	<i>Pinus ponderosa</i>	<i>Pseudotsuga menziesii</i>
biomass	¹ - 1.80 (0.91)	- 15.6 (0.12)	- 25.0 (0.35)	+ 10.1 (0.51)	- 14.0 (0.48)
height	+ 3.3 (0.85)	+ 23.4 (0.78)	+ 23.8 (0.71)	+ 8.9 (0.76)	- 18.6 (0.31)
diameter	+ 21.6 (0.25)	+ 8.3 (0.79)	- 31.7 (0.72)	² + 10.1 (0.01)	- 18.2 (0.27)
root	+ 5.3 (0.63)	+ 5.7 (0.47)	- 13.9 (0.32)	+ 7.1 (0.27)	- 17.3 (0.39)
stem	- 9.6 (0.17)	- 8.7 (0.38)	+ 14.1 (0.30)	+ 1.3 (0.89)	- 14.2 (0.41)
old needles	- 7.1 (0.51)	- 17.3 (0.35)	- 11.1 (0.67)	- 50.2 (0.23)	- 17.8 (0.46)
new needles	+ 17.0 (0.62)	² - 15.5 (0.01)	+ 27.0 (0.25)	+ 19.5 (0.28)	- 8.8 (0.57)

B. 1989 October Harvest

	<i>Abies concolor</i>	<i>Abies lasiocarpa</i>	<i>Picea engelmannii</i>	<i>Pinus ponderosa</i>	<i>Pseudotsuga menziesii</i>
biomass	² + 29.5 (0.01)	0.0 (0.99)	- 10.3 (0.61)	+ 50.7 (0.10)	+ 10.0 (0.30)
height	+ 11.6 (0.10)	+ 12.0 (0.36)	+ 8.2 (0.56)	+ 26.9 (0.16)	+ 11.1 (0.23)
diameter	² + 14.9 (0.03)	+ 0.8 (0.91)	- 3.2 (0.78)	+ 9.8 (0.09)	+ 19.3 (0.23)
root	- 3.5 (0.77)	- 13.8 (0.10)	² + 8.6 (0.23)	+ 7.2 (0.63)	+ 0.5 (0.96)
stem	+ 14.4 (0.28)	+ 11.4 (0.15)	- 4.6 (0.37)	- 7.7 (0.32)	+ 7.0 (0.52)
old needles	- 21.9 (0.27)	+ 18.9 (0.29)	- 14.3 (0.44)	+ 3.3 (0.89)	² - 22.8 (0.02)
new needles	² + 23.4 (0.40)	- 59.8 (0.38)	- 5.3 (0.71)	- 26.3 (0.86)	+ 10.8 (0.81)

¹ Parenthetical values are the probability that the change is significant between treatments (chamber means of six seedlings were the replicates, df=1). Bold indicates values significant at p<0.05.

² Greater allocation to a tissue type in elevated ozone versus charcoal filtered chambers.

Table 5—Evaluation of statistical significance for CO₂ exchange from figure 1. The differences between treatment means or the number of chambers that would have been required for the means to have been significantly different at the p=0.05 level have been calculated, assuming the same population variance.

Species	p ¹	Date of comparison	Difference in mean required	No. of chambers required
CO₂ exchange				
<i>Abies concolor</i>	(0.77)	9/22	1.8x	500
<i>Abies lasiocarpa</i>	(0.99)	9/22	5.2x	330
<i>Picea engelmannii</i>	(0.70)	9/19	4.0x	190
<i>Pinus ponderosa</i>	(0.27)	8/10	1.1x	2
<i>Pseudotsuga menziesii</i>	(0.75)	9/21	3.5x	140

¹ Parenthetical values are probability of significant differences between gas exchange values from figure 1.

P. strobus (Barnes 1972), and in different families of *P. ponderosa* (Beyers and others 1992). In addition to genetic differences, plant water status, leaf tissue nutrient content, and different seasonal patterns of gas exchange or phenology may alter individual plant sensitivity to ozone and contribute to the lack of statistical power to detect treatment effects or to resolve discrepancies between published studies.

Acknowledgments

We thank Paul Dunn (planning), Anthony Gomez (installing the fumigation facility, planting), David Herring (computer programming, data acquisition and control systems), Susan Schilling and David Randall (statistical design and analysis), and David Larson (maintaining plants). Douglas Pumphrey (District Ranger, San Jacinto District, San Bernardino National Forest) kindly permitted use of land and water at the fumigation site. This research was supported in part by the Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, as a component of the National Acid Precipitation Assessment Program.

References

- Barnes, R.L. 1972. **Effects of chronic exposure to ozone on photosynthesis and respiration of pines.** Environmental Pollution 3: 133-138.
- Beyers, J.L.; Riechers, G.H.; Temple, P.J. 1992. **Effects of long-term ozone exposure and drought on the photosynthetic capacity of ponderosa pine (*Pinus ponderosa* Laws).** New Phytologist 122: 81-90.
- Byres, D.P.; Johnson, J.D.; Dean, T.J. 1992. **Seasonal response of slash pine (*Pinus elliotii* var. *elliottii* Engelm.) photosynthesis to long-term exposure to ozone and acidic precipitation.** New Phytologist 122: 91-96.
- Bytnerowicz, A.; Grulke, N.E. 1992. **Physiological effects of air pollutants on western trees.** In: Olson, R.K.; Binkley, D., eds. Effects of air pollution on western forests. New York: Springer-Verlag; 183-233.
- Carter, G.A.; Smith, W.K.; Hadley, J.L. 1988. **Stomatal conductance in three conifer species at different elevations during summer in Wyoming.** Canadian Journal of Forest Research 18: 242-246.
- Conard, S.G.; Radosevich, S.R. 1981. **Photosynthesis, xylem pressure potential, and leaf conductance of three montane chaparral species in California.** Forest Science 27(4): 627-639.
- DeLucia, E.H.; Smith, W.K. 1987. **Air and soil temperature limitations on photosynthesis in Engelmann spruce during summer.** Canadian Journal of Forest Research 17: 527-533.
- Draper, N.; Smith, H. 1981. **Applied regression analysis.** New York: John Wiley and Sons, Inc.; 709 p.
- Ernst, W.H.O.; Tonneijck, A.E.C.; Pasman, F.J.M. 1985. **Ecotypic response of *Silene cucubalus* to air pollutants (SO_2 , O_3).** Journal of Plant Physiology 118: 439-450.
- Grulke, N.E.; Miller, P.R.; Wilborn, R.D.; Hahn, S. 1989. **Photosynthetic response of giant sequoia seedlings and rooted branchlets of mature foliage to ozone fumigation.** In: Olson R.K.; Lefohn A.S., eds. Effects of air pollution on western forests. Pittsburgh, PA: Air, Waste, and Water Management Association; 429-441.
- Hanson, P.J.; Samuelson, L.J.; Wullschlegel, S.D.; Tabberer, T.A.; Edwards, G.S. 1994. **Seasonal patterns of light saturated photosynthesis and conductance for mature tree and seedling *Quercus rubra* foliage: differential sensitivity to ozone exposure.** Tree Physiology 14: 1351-1366.
- Heagle, A.S.; Philbeck, R.B.; Rogers, H.H.; Letchworth, M.B. 1979. **Dispensing and monitoring ozone in open-top chambers for plant effects studies.** Phytopathology 69: 15-20.
- Hinckley, T.M.; Schaap, W.; Wang, D. 1990. **Rapid recovery of photochemical efficiency of PSII in Douglas-fir following ozone treatment.** Ecological Society of America Bulletin 71(2): 189.
- Hogsett, W.E.; Tingey, D.T.; Hendricks, C.; Rossi, D. 1989. **Sensitivity of western conifers to SO_2 and seasonal interactions of acid fog and ozone.** In: Olson R.K.; Lefohn A.S., eds. Effects of air pollution on western forests. Pittsburgh, PA: Air, Waste, and Water Management Association; 469-492.
- Lefohn, A.S.; Hogsett, W.E.; Tingey, D.T. 1986a. **A method for developing ozone exposures that mimic ambient conditions in agricultural areas.** Atmosphere and Environment 20: 361-366.
- Lefohn, A.S.; Hogsett, W.E.; Tingey, D.T. 1986b. **The development of sulfur dioxide and ozone exposure profiles that mimic ambient conditions in the rural southeastern United States.** Atmosphere and Environment 21: 659-669.
- Patterson, M.T.; Rundel, P.W. 1989. **Seasonal physiological responses of ozone-stressed Jeffrey pine in Sequoia National Park, California.** In: Olson R.K.; Lefohn A.S., eds. Effects of air pollution on western forests. Pittsburgh, PA: Air, Waste, and Water Management Association; 419-427.
- Samuelson, L.J.; Kelly, J.; Mays, P.A.; Edwards, G.S. 1996. **Growth and nutrition of *Quercus rubra* L. Seedlings and mature trees after three seasons of ozone exposure.** Environmental Pollution 91: 317-323.
- Taylor, G.E., Jr. 1978. **Genetic analysis of ecotypic differentiation within an annual plant species, *Geranium carolinianum* L., in response to sulfur dioxide.** Botanical Gazette 139: 326-368.
- Thornley, J.H.M. 1976. **Mathematical models in plant physiology.** London: Academic Press; 318 p.
- Weber, J.A.; Clark, C.S.; Hogsett, W.E. 1993. **Analysis of the relationships among O_3 uptake, conductance, and photosynthesis in needles of *Pinus ponderosa*.** Tree Physiology 13: 157-172.