Sensitivity of Selected Western Conifers to Ozone

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ABSTRACT

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Seedlings of 11 conifer species and two hybrids were fumigated in outdoor chambers with 0.36 ppm ozone, 12 hr/day for 37 ± 7 days. Temperatures and relative humidities experienced by the 2- to 3-yr-old container-grown seedlings were similar to the natural forest environment of these species. The *Pinus jeffreyi* × *P. coulteri* hybrid and *P. monticola* were the most sensitive. The order of decreasing sensitivity of the others was *P. ponderosa, P. jeffreyi, Abies concolor, P. coulteri, A. magnifica, P. radiata* × *P. attenuata, P. attenuata, Callocedrus decurrens, Pseudotsuga macrocarpa, Pinus lambertiana,* and *P. ponderosa* var. *scopulorum*.

Summaries that categorize the relative ozone sensitivity of conifer species as sensitive, intermediate, and tolerant based on visible foliage injury show that about 25% of 31 species are rated sensitive (1,5). Much of this information is derived from an ozone fumigation study of 18 species native to the eastern United States (2), and some is based on observations of large trees in the field (3,4). Seedling populations, however, provide a more comparable estimate of ozone sensitivity than populations of field trees that have been subject to selection by diseases, insects, and abiotic agents.

This paper reports a screening of 11 conifer species and two hybrids native to California to determine their relative sensitivity to ozone at the seedling stage of development.

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MATERIALS AND METHODS

During January in three consecutive years, 100–200 1- or 2-yr-old bareroot seedlings nursery-grown from seed lots collected in the Southern California mountains or southern Sierra Nevada mountains were transplanted, one to a container (10 cm diameter × 35 cm high). The species were *Pinus monticola* Dougl., *P. ponderosa* Laws., *P. jeffreyi* Grev. & Balf., *P. coulteri* D. Don., *P. attenuata* Lemm., *P. lambertiana* Dougl., *Abies concolor* (Gord. & Glend.) Lindl., *A. magnifica* A. Murr., *Pseudotsuga macrocarpa* Mayn., and *Callocedrus decurrens* Torr. Florin. Hybrids included *Pinus jeffreyi* × *P. coulteri* and *P. radiata* D. Don. × *P. attenuata*. Seedlings of *P. ponderosa* var. *scopulorum* Engelm. were obtained from the state nursery at Fort Collins, CO. The rooting medium was a 2:1:1 mixture of washed plaster sand:peat moss:coarse vermiculite; the mixture was amended with lime and gypsum and fumigated with methyl bromide. Each container was fertilized early each spring with one 3-g NPK (14-4-6) fertilizer tablet (Agriform International Chemicals, Inc., Newark, CA 94560). Seedlings were grown in charcoal-filtered air greenhouses for about one and one-half growing seasons, then fumigated in eight mylar-covered chambers (0.9 × 0.9 × 1 m) located outside the greenhouses.

Six plants of each of four species were randomly distributed in each chamber. Chambers were exposed to full sunlight filtered through 40% shade cloth from 10 a.m. to 4 p.m.; they were fully shaded during the remainder of the daylight period. Refrigeration coils were placed in the air intake to control high midday temperatures. Representative temperature ranges during the daily fumigation periods were 20–30 C (July), 22–31 C (August), 22–27 C (September), and 13–22 C (October). The corresponding ranges for relative humidity were 48–65% (July), 48–58% (August), 35–44% (September), and 52–65% (October). Seedlings were watered manually once or twice each week, depending on prevailing temperatures, with a water drip-type system (Chapin Watermatic, Waterton, NY 13601).

Fumigation chambers received carbon-filtered air from a common manifold. Ozone was generated from tank oxygen by electrical discharge and metered with precision needle valves through flow meters and Teflon tubing to the filtered air inlet of seven chambers; the eighth chamber was a filtered air control. Inside each chamber, the ozone-air mixture was distributed from an overhead perforated distribution manifold (5 cm diameter × 100 cm long) to the foliage of the seedlings and through a perforated (pegboard) floor that separated the foliage from the root containers. A hole at the bottom of the rear wall allowed air to be vented from the chamber. Exhaust velocity was measured with a Biram's-type recording anemometer, and the airflow rate in the chambers was controlled at 1.2 changes per minute by gate valves at the inlet of each chamber.

In each of 3 yr, ozone fumigation was started in the third week of June, when the current-year needles of the greenhouse-grown plants had attained at least three-fourths of their mature length. Fumigation lasted 37 ± 7 days. Seedlings were exposed to 0.36 ppm (707 $\mu g/m^3$) ozone for 12 hr daily. This ozone concentration was selected to place maximum stress on the plants when testing for tolerance or susceptibility. A timer switched the ozone generator and an oxygen solenoid valve on and off daily. Ozone concentration was monitored continuously in one chamber and serially in the remaining fumigation chambers three times each week. The continuous monitoring was rotated from chamber to chamber on each of the 3 days after serial monitoring. Only minor adjustments were required to maintain the desired ozone concentration at ± 10%.

Ozone monitors (Mast Model 724-2) were calibrated at least every 30 days, using the neutral buffered potassium iodide standard method. This calibration includes the 20% downward correction required by the latest official calibration method, which employs an ultraviolet ozone photometer as a transfer standard. An additional small positive correction was required to account for the difference in atmospheric pressure between the calibration laboratory (304 m) and the fumigation site (1,676 m).

Nine fumigations were completed during the three summers. *P. ponderosa* was included in each fumigation. The more important species comprising the California mixed-conifer and pine cover types that were fumigated repeatedly were *P. coulteri, P. jeffreyi, P. lambertiana, A. concolor,* and *C. decurrens.* Species fumigated only once were *P. monticola, P. attenuata, P. radiata* × *P. attenuata, P. jeffreyi* × *P. coulteri, A. magnifica* and *Pseudotsuga macrocarpa.*

Ozone injury symptoms were determined every 10–12 days during the fumigation period by examining the entire complement of current-year and 1-yr-old needles of each seedling. The sequence of symptom development usually started with chlorotic mottle, followed by necrosis and needle abscission. To document the progressive accumulation of injury to needles of both ages, chlorotic mottle and necrosis symptoms were each assigned one of four ordinal values: 0 = no injury, 1 = very slight, 2 = slight, 3 = moderate, and 4 = severe. The tendency for needle abscission could be distinguished as one of three levels: 0 = none, 2 = easily pulled from the stem, and 4 = falling from the stem with no pull required. Six ordinal values—three for each needle age—were summed to obtain an aggregate score for each seedling. The maximum possible score for each seedling was 24.

Distributions of score values were normalized by transformation to common logarithms. Development of injury symptoms in relation to time was analyzed by linear regression, using the pooled data for all fumigations of a single species. Although the length of fumigations averaged 37 ± 7 days, it was feasible to compare responses of species from regression lines after 25 days of exposure. The variances of the injury scores were computed after 25 days of fumigation on the basis of the regression analysis. The effect of month and year during which each fumigation took place was not considered because our principal objective was to observe the relative sensitivity of species.

Table 1. Mean ozone injury scores of 11 conifer species and two hybrids after exposure to 0.36 ppm ozone, 12 hr daily, for 25 days

Species or hybrid	**Mean log injury score (P = 0.05)**[a]
Pinus jeffreyi × *P. coulteri*	1.24 ± 0.10
P. monticola	1.24 ± 0.15
P. ponderosa	1.00 ± 0.05
P. jeffreyi	0.97 ± 0.05
Abies concolor	0.91 ± 0.10
P. coulteri	0.87 ± 0.10
A. magnifica	0.69 ± 0.10
P. radiata × *P. attenuata*	0.69 ± 0.10
P. attenuata	0.51 ± 0.15
Callocedrus decurrens	0.51 ± 0.06
Pseudotsuga macrocarpa	0.41 ± 0.10
Pinus lambertiana	0.38 ± 0.05
P. ponderosa var. *scopulorum*	0.28 ± 0.15

[a] Range = 0–1.38; species with values below 0.48 were considered tolerant.

RESULTS

All species developed similar symptoms, even though secondary leaf morphology varied from long thin needles with three surfaces (*P. ponderosa, P. jeffreyi,* and *P. coulteri*) to short needles with two surfaces (*A. concolor*) to small scalelike leaves closely appressed to branchlets (*C. decurrens*). In all species, chlorotic mottle developed first on leaves 1 yr or older, and then on the youngest foliage. After chlorotic mottle had spread from the tip so that one-third to one-half of a needle was uniformly yellow, a tan necrosis of tip tissue developed toward the needle base. In some cases, necrosis appeared without prior development of chlorotic mottle at the needle tip or appeared as an irregular spot or band. As mottle and necrosis intensified, the 1-yr and older needles began to abscise until, in the case of sensitive individuals, only the current-year needles remained. Abscission of current year needles occurred only occasionally.

Regression analysis showed that all species began to show injury symptoms by 6 days after fumigation began. The means and 5% confidence bands associated with the least-squares linear fit for the species and hybrids were used to show relative sensitivity to ozone (Table 1). The *P. jeffreyi* × *P. coulteri* hybrid and *P. monticola* were the most sensitive. Among the species comprising California mixed-conifer forest and associated woodland cover types, *P. ponderosa* and *P. jeffreyi* were about equally sensitive, followed by *A. concolor, P. coulteri, C. decurrens* or *P. attenuata, Pseudotsuga macrocarpa,* and *Pinus lambertiana.*

The proportion of seedlings of each species that survived ozone fumigation with only slight injury (log injury score less than 0.48) indicates the probability of obtaining sufficient quantities for outplanting in chronically polluted areas. For the five species that make up the mixed-conifer forest type in southern California, 5.6% of *P. ponderosa*, 8.2% of *A. concolor*, 14.5% of *P. jeffreyi*, 46.7% of *C. decurrens*, and 62.2% of *P. lambertiana* seedlings were in this category.

DISCUSSION

P. ponderosa and *P. monticola* were the most sensitive species in our study and that of Wilhour and Neely (6), who measured visible injury and growth of nine conifer species exposed to 0.10 ppm ozone, 6 hr per day, every day for 18 wk. *P. ponderosa* showed the most foliar injury (20%) in their experiment. *P. lambertiana*, which had 1% injury in Wilhour and Neely's study, was one of the species least injured in our study (Table 1). Our results were inconsistent with theirs, however, in the case of *P. jeffreyi*, which showed injury to only 2% of the foliar surface, and *P. monticola*, which had no symptoms.

Wilhour and Neely (6) found discrepancies between the severity of visible symptoms and growth. For example, there were significant reductions in growth for only *P. ponderosa* and *P. monticola*; however, visible symptoms of injury were obvious on *P. ponderosa* and inconspicuous on *P. monticola*. This discrepancy demonstrates the need for measured growth variables for assessing the effects of chronic ozone exposure.

On the basis of visible ozone symptoms, the range of sensitivity is broad among the species and hybrids and within each population. The relative order of sensitivity determined from these seedling populations compares favorably with field observations (3,4). Among those species native to the California mixed-conifer forest cover type, only *A. concolor* appears to be less sensitive in the field than in our study.

C. decurrens and *P. lambertiana* populations appear to have sufficient numbers of tolerant individuals so that they may be planted with reasonable success in ozone-affected forests. Where site conditions are unfavorable for the establishment of these species, it is important to have the option of planting ozone-tolerant individuals selected from *P. ponderosa* and *P. jeffreyi* populations. It may be feasible to screen populations of the sensitive species in one summer by growing them at existing nursery facilities where ozone concentrations are consistently high. This practice could provide sufficient numbers of ozone tolerant *P. ponderosa, P. jeffreyi*, and *A. concolor* for replanting. More stringent standards should be used when selecting individual trees for future breeding programs aimed at increasing ozone tolerance than for trees to be used only for outplanting. The only trees that should be included are the few that have no detectable injury symptoms after ozone exposure.

Comparing seedling reactions with those of older trees in the field is sometimes risky. In our study, the comparison is feasible because all seedlings had a minimum of two annual complements of secondary needles, which developed the same symptom pattern observed in the field. It would be unwise, however, to use mean injury scores of the seedlings as a quantitative expression of the relative response of older trees exposed under field conditions. The results are a qualitative or relative index of the expected foliage injury to these species, and should be useful for accomplishing field surveys and guiding research on remedial techniques.

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