Ozone Effects on Seedlings of Rocky Mountain Ponderosa Pine

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ABSTRACT

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Two-year-old seedlings of Rocky Mountain ponderosa pine (*Pinus ponderosa* var. *scopulorum*) were fumigated in greenhouse chambers at 0.50 (980 $\mu g/m^3$), 0.40 (784 $\mu g/m^3$), 0.30 (588 $\mu g/m^3$), or 0.25 (490 $\mu g/m^3$) ppm from 0800 to 1800 hours for 21 days. Seedlings fumigated at 0.50, 0.40, and 0.30 ppm showed mottling and chlorosis of needles. No visual symptoms were observed on seedlings fumigated at 0.25 ppm. Compared with untreated controls, 4-yr-old seedlings fumigated at 0.50 ppm ozone from 0900 to 1800 hours for 18 days had significantly depleted chlorophyll contents in each of four age classes of needles.

The Denver metropolitan area of Colorado has a serious air-pollution problem, averaging more than 150 days a year with air quality rated poor or worse (3). Poor days are those when any monitoring site exceeds 100 PSI (Pollutant Standards Index). One of the major pollutants of concern is ozone. In 1980, eight violations of National Ambient Air Quality Standards for ozone were recorded at six of 10 sites where ozone is monitored in the Colorado Front Range (2). The highest ozone concentration in 1980 in Denver was 0.165 ppm, and the highest recorded concentration was 0.375 ppm in 1974 (3).

Ozone concentrations are sufficient in and around Denver to elicit plant response (1,10). James and Staley (10) conducted surveys within and adjacent to the Denver metropolitan area to detect air-pollution injury on ponderosa pine and found that some of the trees displayed symptoms typical of oxidant air pollution. They concluded that controlled ozone fumigation experiments coupled with an expanded ozone monitoring program would be needed to identify injury thresholds and to determine if symptoms observed on ponderosa pine foliage are caused by oxidant air pollution.

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The West Coast variety of ponderosa pine (*Pinus ponderosa* Dougl. ex P. Laws & C. Laws. var. *ponderosa*) is highly susceptible to injury by oxidant air pollutants (13,15,18). Limited work has been conducted to determine whether the Rocky Mountain variety of ponderosa pine (*P. ponderosa* var. *scopulorum* Engelm.) is also sensitive to ozone (16). This paper reports on the sensitivity of Rocky Mountain ponderosa pine seedlings to controlled ozone fumigations.

MATERIALS AND METHODS

Two- and 4-yr-old Rocky Mountain ponderosa pine seedlings were used in the study. Two-year-old seedlings were grown from seeds collected in the Arapahoe, Roosevelt, Pike, and San Isabel national forests of Colorado. Seeds were soaked in water for 24 hr, then planted in plastic tubes (4 × 21 cm) containing soil, vermiculite, and perlite (4:1:1, v/v). Planting was staggered to produce seedlings that were the same age and had elongating needles at the time of fumigation. The tubes were placed in a greenhouse, where they received ambient air. Four-year-old seedlings from the same seed source were donated by the Colorado State Forest Service Foothills Nursery. Seedlings were watered as needed and fertilized with a liquid solution (3.25 ml/L) of 30-10-10 (NPK) fertilizer once a week throughout the growing season (April–October).

Two exposure chambers were constructed with 8-mil polyvinyl chloride plastic film. The chamber design of Heagle et al (8) was used and modified with a hood and vent apparatus to permit fumigations in a greenhouse. The cylindrical chambers were 1.5 m high and 1 m in diameter. The lower half of each chamber was covered by a double layer of film; the inner layer was perforated with 108 holes 1 cm in diameter. Air was introduced through a wooden box containing a blower, a fiberglass filter 10 cm thick, and a charcoal filter 2.5 cm thick. Ozone or charcoal-filtered air was introduced into the chamber via the lower bilayer of plastic. Ozone was generated from reconstituted air (nitrogen and oxygen) by electric discharge with an ozone generator (model 03V5-0, Ozone Research and Equipment Corp., Phoenix, AZ 86019). Ozone was monitored continuously in the fumigation chamber with a Dasibi ozone monitor (model 1003 AH, Dasibi Environmental Corp., Glendale, CA 91205), which was calibrated by the Environmental Protection Agency, Denver. Temperature and relative humidity (RH) were monitored continuously (Thermister, model 701, Yellow Springs Instrument Co., Yellow Springs, OH 45387, and humidity meter, model RH-515-A with HS-3553-B humidity sensor, Hy-Cal Engineering, Santa Fe Springs, CA 90670). All parameters were continuously recorded on a strip-chart recorder (model MS 413B, Esterline Angus Instrument Corp., Indianapolis, IN 46224).

The first experiment was designed to provide a rough estimate of the symptomatic responses of seedlings to various fumigation conditions. Exposures were conducted at 1, 0.75, 0.50, and 0.25 (±0.05) ppm ozone (0.1 ppm = 196 $\mu g/m^3$) for 24 hr/day until visible symptoms developed. Five seedlings with newly elongated needles were exposed in the fumigation and control chambers for each ozone concentration. Fumigations were conducted during April–July 1981. Seedlings were watered and examined each day for symptoms. Temperature and relative humidity could not be held constant but were similar between control and fumigation chambers. The mean hourly temperature of the chambers (± one standard deviation) was 24.6 (±6.1) C and the mean RH was 62.4 (±12.3)%.

The second experiment was designed to determine the threshold ozone concentration for visible injury to seedlings. Exposures were conducted at 0.50, 0.40, 0.30, or 0.25 ppm ozone from 0800 to 1800 hours for 21 consecutive days during July–October 1981 and in May 1982. For each exposure concentration, 20 seedlings were fumigated and

20 were used as controls. The plants stayed in the fumigation chambers during the nonfumigation period. Half of the seedlings in each chamber were grown from seed collected in the Pike and San Isabel national forests and the other half were grown from seeds collected in the Arapahoe and Roosevelt national forests, which are adjacent to the Front Range metropolitan areas of Colorado. Seedlings were watered and examined for symptoms each day before ozone exposure. Each seedling was rated for mottling and chlorosis, the two most prominent symptoms. Four severity classes were used for mottling: no response, slight, moderate, and severe. Three classes were recognized for chlorosis: no response, moderate, and severe. Temperature and relative humidity could not be held constant but were similar between control and fumigation chambers. The mean temperature of the chambers was 23.5 (±4.5) C, and the mean RH was 64 (±8)%.

Chlorophyll was extracted from each year's needles of 4-yr-old seedlings fumigated at 0.50 ppm ozone from 0900 to 1800 hours for 18 days. Four needle years were represented on each seedling. Extractions were also made from unfumigated seedlings. Two repetitions were performed for each needle age. Extractions were performed 3 and 9 days after seedlings were fumigated. Extraction techniques used were those described by Comar (4) and Comar et al (5), except 2.5 g of fresh leaf tissue was ground in a mortar with 85% acetone to which a small amount of calcium carbonate had been added. The mixture was vacuum-filtered through Whatman No. 1 filter paper. The residue was ground again in 85% acetone until a white mash remained, and this was refiltered. The two filtrates were combined and adjusted to a standard volume with 85% acetone. The optical density at 655 μ was immediately measured with a Bausch & Lomb Spectronic 20 spectrophotometer. The chlorophyll concentrations of fumigated and control seedlings were calculated. Concentrations, expressed as percentage of controls, were compared between needle age and interval from fumigation to extraction with a two-way analysis of variance. Comparisons among needle ages were performed using LSDs ($P = 0.05$). Confidence intervals were used to compare effects of ozone concentrations in relation to times of extraction.

RESULTS

Fumigations at 1.00 or 0.75 ppm ozone for 48 hr induced needle banding, tip necrosis, chlorotic mottling, chlorosis of entire needles, and needle abscission. Only mottling and chlorosis were noted after 8 days of fumigation at 0.50 ppm. No seedlings were symptomatic after 21 days of continuous fumigation with 0.25 ppm ozone.

The threshold ozone concentration for visual injury apparently was between 0.25 and 0.30 ppm for 10 hr/day with 21 days of exposure (Table 1). Concentrations of 0.30–0.50 ppm produced chronic chlorotic mottling and general chlorosis, which eventually led to needle abscission. These symptoms were least severe on seedlings exposed to 0.30 ppm ozone (Table 1). No difference in response was noted between the two seed sources; therefore, the results were combined. Mottling or chlorosis was not observed on any control seedlings.

Response time, or time from start of fumigation until symptom expression, also implicated 0.25–0.30 ppm ozone as the threshold concentration for visible injury (Figs. 1 and 2). Mottling (Fig. 1) was the most prominent response and it occurred sooner than general chlorosis (Fig. 2). Mottling and chlorosis developed least rapidly at 0.30 ppm and most rapidly at 0.50 ppm. The proportion of seedlings symptomatic at the end of the 21-day fumigation increased with excessively higher ozone concentrations.

The amount of chlorophyll extracted from needles of seedlings fumigated at 0.50 ppm (9 hr/day, 0900–1800 hours, for 18 days) was related to the intensity of chlorotic mottling and chlorosis of all four age classes of needles (Table 2). Damage on needles within each year ranged from mild chlorosis and mottling to severe chlorosis. Chlorophyll depletion in injured needles within 3 days after the end of the fumigation period was significant compared with controls for all needle ages except the 2-yr-old needles. After six additional days, reductions in chlorophyll were significant for all four needle ages. Chlorophyll concentrations at 9 days were significantly less than concentrations at 3 days for all four needle ages.

DISCUSSION

Rocky Mountain ponderosa pine seedlings are sensitive to certain ozone concentrations. Concentrations of ozone at 0.30–0.50 ppm induced chlorotic mottling visibly similar to that found on ponderosa pine growing under natural conditions in the forests of southern California (13,15). The loss of chlorophyll

Table 1. Percentage of seedlings displaying different degrees of mottling and chlorosis when fumigated at four ozone concentrations for 21 days.[a]

Ozone concentration (ppm)[b]	Percentage of seedlings displaying symptoms[c]						
	Mottling				Chlorosis		
	None	Slight	Moderate	Severe	None	Moderate	Severe
0.50	5	70	25	0	20	65	15
0.40	0	70	25	5	30	60	10
0.30	50	45	5	0	70	25	5
0.25	100	0	0	0	100	0	0
Control	100	0	0	0	100	0	0

[a] Fumigations conducted 10 hr/day (0800–1800 hours). Symptoms recorded on the 21st day.
[b] All ozone concentrations were ±0.05 ppm.
[c] n = 20 For each ozone concentration.

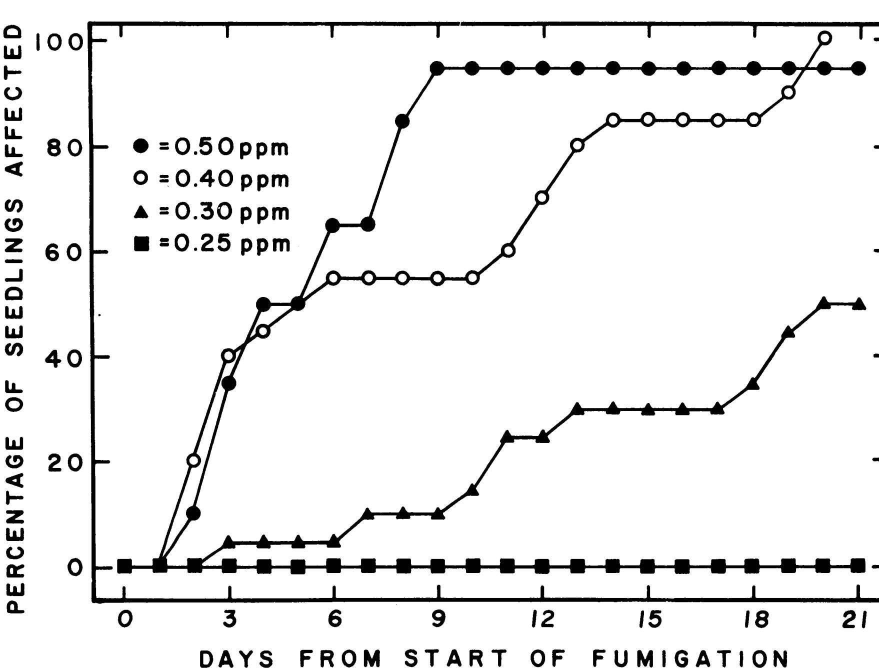

Fig. 1. Needle mottling on ponderosa pine seedlings fumigated at four ozone concentrations. Fumigations conducted from 0800 to 1800 hours for 21 days.

Table 2. Chlorophyll concentrations, expressed as a percentage of unfumigated controls, from needles of 4-yr-old Rocky Mountain ponderosa pine seedlings fumigated at 0.50 ppm ozone from 0900 to 1800 hours for 18 days

Needle age	Chlorophyll concentrations 3 and 9 days after removal from fumigation chamber[z]	
	3	9
1982 (current year)	82.5 a	69.1 a
1981 (1 yr old)	77.9 b	67.4 a
1980 (2 yr old)	94.2 a	73.7 a
1979 (3 yr old)	85.8 a	65.1 a
LSD	13.6	13.6
Mean	85.1	68.8

[z] Means followed by same letter not significantly different ($P = 0.05$), n = 2, on each sampling day; chlorophyll concentrations expressed as a percentage of that year's unfumigated controls. The confidence interval used to compare concentrations between the times of extractions and between fumigated and control needles was ±6.9.

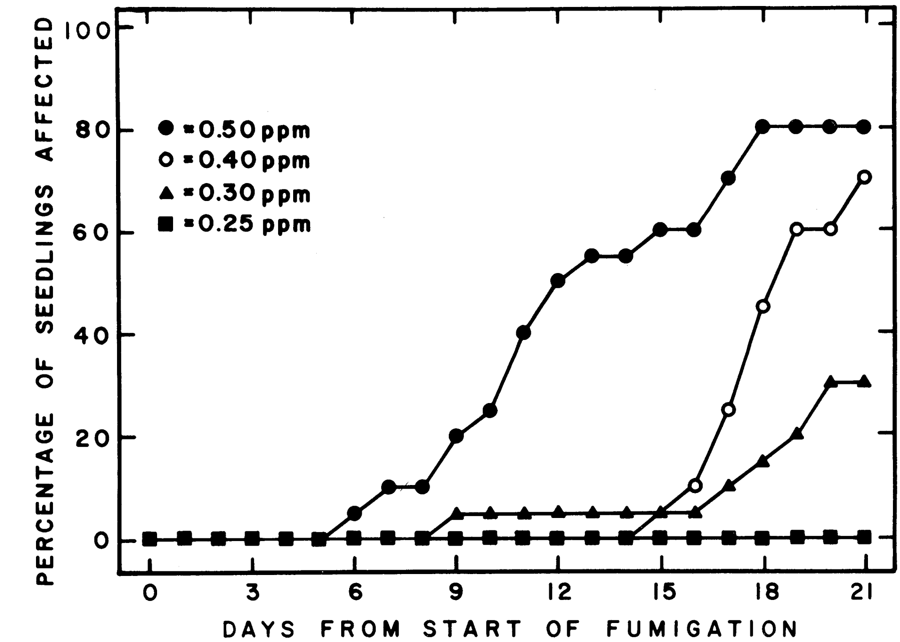

Fig. 2. Chlorosis on seedlings of ponderosa pine fumigated at four ozone concentrations. Fumigations conducted from 0800 to 1800 hours for 21 days.

as indicated by mottling and chlorosis was confirmed by the results of chlorophyll extractions as previously shown in beans (17) and pines (13).

Needle age has been reported as an important factor in determining sensitivity of pine needles to ozone. Conflicting results have been reported. Davis and Wood (6) studied the relative susceptibility of 18 coniferous species to ozone and found that current-year foliage was more sensitive than older foliage. In contrast, Miller and Vandoren (14) reported that 1-yr-old needles of West Coast ponderosa pine were injured more severely than current-year needles. In our study, 1-yr-old needles appeared more sensitive on the basis of chlorophyll extraction at 3 days but not at 9 days after fumigation.

The threshold for visible injury on Rocky Mountain ponderosa pine apparently lies between 0.25 and 0.30 ppm for a fumigation of 10 hr/day for 21 days. This does not imply that ozone concentrations less than 0.30 ppm were innocuous or that seedlings without visible symptoms after a 21-day fumigation were unaffected. Miller et al (12) showed decreases in the rate of apparent photosynthesis during fumigation of West Coast ponderosa pine with 0.15 or 0.30 ppm ozone, indicating functional impairment before chlorotic mottling developed. Evans and Miller (7) found that histological and histochemical changes in West Coast ponderosa pine occurred within 5–7 days, but visible symptoms were not evident until 2–3 wk after ozone fumigation at 0.45 ppm.

It has been suggested that the Rocky Mountain variety of ponderosa pine may be more resistant to oxidant injury than the coastal variety (10). It is not possible, from the results of our study, to ascertain whether this is the case. Many variables, including plant age, environmental conditions, and experimental design, make comparison with previous field and laboratory studies difficult (9). It is interesting, however, that results similar to ours were observed on West Coast ponderosa pine, specifically that typical chlorotic mottling appeared on needles after fumigating 3–4 wk with 0.30 ppm ozone for 9 hr/day (11).

Field surveys of ponderosa pine within and adjacent to the Denver metropolitan area during 1978 and 1979 found few trees showing symptoms typical of oxidant air pollution (10). Most injured trees were southeast of Denver. Symptoms observed included shortened needles, fewer needles, and chlorotic banding. No chlorotic mottling typical of seedlings fumigated in this study or oxidant-damaged trees in California was found. Whether oxidant pollutants generated in the Denver metropolitan area are reaching forested areas is not known but is strongly suspected. Although diurnal wind patterns typical of mountain slopes may cause pollutants to reach the forested areas west of Denver (10), prevailing southwesterly winds tend to carry most pollution east, away from ponderosa pine stands. The potential for oxidant damage to ozone-sensitive vegetation therefore appears to be greatest within and east of the metropolitan areas along the Front Range. Only after an expanded monitoring program is employed, coupled with field fumigations on ponderosa pine, will it be possible to make valid conclusions regarding the potential for air-pollution damage to Rocky Mountain ponderosa pine.

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