Osmotic adjustment induced by elevated ozone: interactive effects of acid rain and ozone on water relations of field-grown seedlings and mature trees of *Pinus ponderosa*

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**Summary** We investigated the effects of simulated acid rain and elevated ozone on tissue water relations of mature clones of a fast-growing genotype of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) and their half-sib seedlings. Whole seedlings and branches of mature trees were exposed to acid rain (pH 5.1 and 3.0) and ozone (ambient and twice-ambient) treatments in open-bottomed chambers. The acid rain treatment was applied to foliage weekly from January to April 1992. The ozone treatment was applied daily from September 1991 to November 1992. The treatments had little effect on the water relations of branches of mature trees. In contrast, water relations of seedlings were affected by the treatments, particularly by the twice-ambient ozone treatment, and in many instances, there was a significant acid rain × ozone interaction. A combination of twice-ambient ozone and pH 3.0 rain lowered seedling solute potential, turgor loss point and cell-wall elasticity but increased pressure potential and symplastic water content, whereas total water content was unchanged. We conclude that twice-ambient ozone caused osmotic adjustment in seedlings, and the response was magnified by pH 3.0 rain. The long-term consequences of this response are unclear because although osmotic adjustment and increased pressure potential might be advantageous to seedlings in resisting drought and maintaining growth, the concurrent decrease in cell-wall elasticity might induce adverse effects. We note that the effects of elevated ozone on water relations of ponderosa pine seedlings were similar to the effects of drought on water relations of many species.

**Keywords:** branches, cell wall elasticity, drought, pH, ponderosa pine, pressure potential, solute potential, symplastic water content, turgor loss point.

**Material and methods**

**Site**

Field studies were conducted at the USDA Forest Service Chico Tree Improvement Center (CTIC) in Chico, CA. The site is located at the lower elevation of the natural range of ponderosa pine. The climate is Mediterranean with hot dry summers and mild wet winters.

**Plant material**

Plants consisted of field-grown, grafted mature trees and corresponding half-sib seedlings of ponderosa pine, Clone 3087. This clone was selected by the USDA Forest Service in the mid-1970s to produce seed for future plantations in California.

Elevated tropospheric ozone and acid rain are among the air pollutants that can cause severe damage to terrestrial and aquatic ecosystems (Miller et al. 1977, Hileman 1984, Chappelka et al. 1985, Reich et al. 1986, Pye et al. 1988, USNAPAP 1991). It has been hypothesized that acid rain enters plant cells through the cuticle of the leaf surface, alters the permeability of cell membranes, and produces osmotically active anions leading to decreased cell solute potential (Heath 1980). Ozone has been reported to cause a decrease in relative water content (Evans and Ting 1974), no significant changes in total water potential, solute potential and pressure potential of beech seedlings (Taylor and Dobson 1989), a temporary increase in cell solute potential of water-stressed Norway spruce (*Picea abies* (L.) Karst.) seedlings (Dobson et al. 1990), an increase in total water potential of cotton (Temple 1990), and a decrease in total water potential of orange trees (Olszyk et al. 1991). The conflicting results obtained from studies of the effects of ozone on plant water relations may be related to the limited number of water relations components examined or to the use of short-term exposure to ozone under laboratory conditions, or both.

We investigated the long-term interactive effects of simulated acid rain and twice-ambient ozone on total water potential (\(\Psi_w\)), solute potential (\(\Psi_s\)), pressure potential (\(\Psi_p\)), turgor loss point (TLP), bulk modulus of elasticity (\(\varepsilon\)), and total water content (\(V_t\)) and its osmotically active portion, symplastic water content (\(V_s\)), of foliage of mature clones of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) and their half-sib seedlings growing under field conditions.
In the mid-1970s, scions were collected from a 70-year-old tree growing at about 1200 m elevation on the El Dorado National Forest in the central Sierra Nevada (USDA-FS Seed Zone 526). Scions were grafted onto 3-year-old root stocks of unknown genetic origin and transplanted to a seed orchard in 1980. In 1990, platforms were constructed around the grafted trees (6- to 7-m tall) to facilitate access. In 1992, at the time of experimental measurements, the grafted trees possessed morphological characteristics of 80-year-old mature trees as indicated by branch diameter, branch angle, needle length, bark color and cone production.

Seed was collected at the same time as scions from the same parent tree, stored until April 1989, and then grown in containers in a greenhouse. In February 1990, seedlings were transplanted to a site adjacent to the grafted mature trees.

After transplanting, both mature trees and seedlings were watered every other week from May to October of both 1991 and 1992, and competing herbaceous species were removed regularly.

Acid rain and ozone treatments

Air pollution treatments were applied in branch exposure chambers (Houpis et al. 1991), which were installed in September 1991. A single chamber covered 3 years of annual shoot growth of an individual branch of a mature tree, whereas each seedling was entirely covered by a single chamber. Chambers were open-bottomed, 1.5 m long × 0.7 m diameter, and were made of 5-mm Teflon sheeting supported by an aluminum frame covered by nonreactive Teflon tape. Teflon was used because it is transparent to longwave heat radiation, does not alter light quality, and is nonreactive to ozone. The chamber included a mixing region at the top, a main exposure region in the middle, and an exhaust frustum at the bottom. A fan connected to the mixing region at the top of the chamber circulated ambient or ozonated air downward and prevented variations in chamber temperature.

Treatments consisted of four combinations of two ozone (ambient and twice-ambient) and two rain (pH 5.1 and 3.0) regimes: (1) pH 5.1 rain and ambient ozone (pH$_{5.1}$ + O$_{amb}$), (2) pH 5.1 rain and twice-ambient ozone (pH$_{5.1}$ + O$_{twice}$), (3) pH 3.0 rain and ambient ozone (pH$_{3.0}$ + O$_{amb}$), and (4) pH 3.0 rain and twice-ambient ozone (pH$_{3.0}$ + O$_{twice}$). Because the pH of natural rain in Sierra Nevada is 5.1 to 5.6, the pH$_{5.1}$ + O$_{amb}$ treatment was considered the control chamber treatment.

Simulated rain regimes were applied weekly during the normal winter precipitation period from January 17 until April 30, 1992, based on records of Leonard et al. (1981) and on 25-year precipitation records from the University of California Blodgett Forest Research Station near Georgetown, CA. A total of 17 rain events, with a deposition of 5 cm per event, resulted in a total deposition of 85 cm. The rain solutions were based on a 2/3 (equivalent basis) mix of H$_2$SO$_4$ and HNO$_3$ plus the kinds and amounts of ions needed to simulate the chemical composition of normal and acidic rains in northern California (McColl and Johnson 1983). Rain droplets with diameters of 0.1 to 1.0 mm were produced with a calibrated nozzle mounted at the top of each branch exposure chamber. The solution throughfall was collected in containers at the base of each chamber.

Twice-ambient ozone was applied daily (0600 to 2000 h in spring and summer, 0800 to 1700 h in fall and winter) from September 1991 to November 1992. Ozone fumigation was interrupted for 3 weeks in mid-December 1991 for system maintenance and calibration, and again for 3 weeks in late June 1992 because of the break-down of the ozone generator. Twice-ambient ozone was generated from compressed ambient air by electrical discharge with an ozone generator (PCI Ozone and Control Systems Inc., West Caldwell, NJ).

Ambient ozone concentration was monitored every 15 min and logged by a data acquisition system that calculated the twice-ambient value and transmitted signals to the ozone generator to produce twice-ambient ozone concentration within the designated chambers. All fumigation and sample gases were passed through Teflon tubing. All sampling instruments were calibrated and serviced according to standard operating procedures (Houpis et al. 1988). Ozone analyzers (Model 1003, Dasibi Environmental Corp., Glendale, CA) were calibrated against a California Air Resources Board certified transfer standard. Midday ambient ozone concentration increased from 0.038 ppm in February to 0.058 ppm in June and August, and then decreased to 0.020 ppm in November 1992. Midday elevated ozone concentration inside the chambers increased from 0.075 ppm in February to 0.115 ppm in June and August, and then decreased to 0.044 ppm in November 1992.

Sampling and measurement techniques

In April, July and August 1992, fully expanded fascicles were harvested 2 and 3 days after scheduled irrigation for seedlings and mature trees, respectively. In April and July, 1-year-old fascicles, and in November, current-year fascicles were examined. Fascicles were collected from the upper part of the growing segments and were pulled so that a short strand remained extended from the base. When pressurizing fascicles, the extended strand prevented resin from obstructing the flow of sap from the xylem. Samples were harvested at predawn to prevent confounding effects of diurnal transpiration and kept on ice in the dark until water relations measurements were made.

Individual fascicles, each with three needles, were placed inside two serially connected pressure chambers (Soil Moisture Equipment Corp., Santa Barbara, CA), and pressurized with nitrogen gas. Bench-drying pressure-volume (P-V) techniques were used to construct P-V curves (Tyree and Hammel 1972, Cheung et al. 1976, Koide et al. 1989). The P-V measurements of all treatment combinations for seedlings or mature trees were made within 8 h.

Data analysis

For seedlings, a $2^2$ factorial experiment within a completely randomized design was used with three replicates per treatment combination. To test for chamber effects, three replicates of nonchambered nontreated seedlings were compared with seedlings in the control (pH$_{5.1}$ + O$_{amb}$) treatment. For mature trees, the design slightly differed from that of seedlings and...
was a $2^2$ factorial experiment within a split-plot, randomized complete block setting. Four mature trees were assigned randomly to either of the two rain regimes. On each tree, three similar branches were selected; two branches were assigned randomly to either of the two ozone regimes, and the third branch was used as a nonchambered nontreated replicate (total of four) to test for chamber effects.

The GLM (General Linear Model) procedure with SS III option of the SAS System (SAS/STAT, SAS Institute Inc., Cary, NC) was used to analyze the data. When rain × ozone interaction was not significant, the main-effect means were compared. When rain × ozone interaction was significant, ANOVA was performed on four simple-effect means, and Student-Newman-Keuls (SNK) test was used for multiple mean comparison (Steel and Torrie 1980). Main- or simple-effect means were declared significant at $P < 0.05$.

Results

There were no significant differences between the control (pH$_{3.0}$ + O$_{amb}$) and nontreated nontreated branches at any measurement date, indicating that the results were not confounded by chamber effects.

Seedlings

Exposure of seedlings to acid rain and twice-ambient ozone alone or in combination had no significant effect on predawn $\Psi_s$ in April, July or November 1992. Water potential values of seedlings in all treatments were near maximum at about $-0.5$ MPa on all measurement dates (Figure 1).

Interactions between rain and ozone effects on $\Psi_s$ of seedlings were significant in April ($P = 0.02$) and July ($P = 0.006$) (Figure 1). In April, the simple-effect means of $\Psi_s$ in the pH$_{3.1}$ + O$_{amb}$ and pH$_{3.1}$ + O$_{twice}$ treatments were not significantly different but they both differed ($P < 0.05$) from the simple-effect means of $\Psi_s$ in the pH$_{5.1}$ + O$_{amb}$ and pH$_{5.1}$ + O$_{twice}$ treatments (Figure 1). Seedlings in the pH$_{3.0}$ + O$_{twice}$ treatment had the lowest $\Psi_s$, whereas seedlings in the pH$_{3.0}$ + O$_{amb}$ treatment had the highest $\Psi_s$. In July, simple-effect means of $\Psi_s$ in the pH$_{3.1}$ + O$_{amb}$ and pH$_{3.0}$ + O$_{amb}$ treatments did not differ significantly but they both differed ($P < 0.05$) from the simple-effect means of $\Psi_s$ in the pH$_{3.1}$ + O$_{twice}$ and pH$_{3.0}$ + O$_{twice}$ treatments. The simple-effect mean of $\Psi_s$ in the pH$_{3.1}$ + O$_{twice}$ treatment was also significantly ($P < 0.05$) lower than the simple-effect mean of $\Psi_s$ in the pH$_{3.0}$ + O$_{twice}$ treatment. Because the acid rain by ozone interaction was not significant in November, we compared main-effect means for this date. The comparison showed that $\Psi_s$ was significantly ($P = 0.014$) decreased in seedlings in the twice-ambient ozone treatment.

Acid rain and ozone effects on $\Psi_w$ of seedlings were of similar magnitude to those on $\Psi_s$, but the direction of the changes was reversed (Figure 1).

Interactions between rain and ozone effects on TLP of seedlings were significant in April ($P = 0.026$) and July ($P = 0.009$). In April, the only significant difference was a lower TLP in seedlings in the pH$_{3.0}$ + O$_{twice}$ treatment compared with seedlings in the pH$_{3.1}$ + O$_{amb}$ treatment. In July, the simple-effect means of TLP were significant in April ($P = 0.051$) and July ($P = 0.001$). The twice-ambient ozone treatment caused a significantly ($P = 0.009$) lower TLP, and the decrease was greater ($P < 0.05$) in the presence of pH 5.1 rain than in the presence of pH 3.0 rain. In November, we compared main-effect means, because the acid rain × ozone interaction was not significant, and found that the twice-ambient ozone treatment caused a significant decrease ($P = 0.009$) in TLP.

In April and November, significant interactions between rain and ozone effects were detected on $\epsilon$ ($P = 0.001$ and 0.007, respectively). The twice-ambient ozone treatment caused $\epsilon$ to decrease, particularly in seedlings exposed to pH 3.0 rain (Figure 1). In July, $\epsilon$ was not significantly affected by acid rain or twice-ambient ozone either alone or in combination.

Acid rain, ozone and a combination of the two treatments had no significant effects on $V_t$ in April, July or November (Figure 2). Because there were no significant acid rain × ozone interactions on $V_t$, we compared the main-effect means (Figure 2). There was no significant effect of acid rain or twice-ambient ozone on $V_t$ in April. In July, $V_t$ increased ($P = 0.004$) in seedlings exposed to twice-ambient ozone, whereas in November, $V_t$ increased in seedlings in both the pH 3.0 rain ($P = 0.047$) and twice-ambient ozone ($P = 0.002$) treatments.
Mature trees

The only significant effects of acid rain and twice-ambient ozone on tissue water relations of mature trees were (1) an increase ($P = 0.028$) in $\epsilon$ in April caused by the main-effect of pH 3.0 rain, and (2) rain $\times$ ozone interaction ($P = 0.038$) on $\epsilon$ in November. There were no significant simple-effect mean differences in November (Figures 3 and 4).

Discussion

Total water potentials of seedlings were near maximum ($-0.5$ MPa) throughout the study and, contrary to reports by Taylor and Dobson (1989), they were not affected by the pollutant treatments. Similar $\Psi_w$ values indicate that the potential effects of water deficit and transpiration were minimized as a result of regular irrigation and the use of predawn measurements, respectively, and that confounding effects of differences in $\Psi_w$ on other water relations components were prevented.

Solute potential of seedlings was decreased by twice-ambient ozone, particularly in the presence of pH 3.0 rain. This finding is contrary to previous reports (Taylor and Dobson 1989, Dobson et al. 1990) and differs from the expectation that elevated ozone increases osmotic potential as a result of a reduction in photosynthesis and, hence, cellular concentration.

It may be argued that $\Psi_s$ decreased as a result of the movement of N ions from the acid rain to leaf cells through the cuticle, or that $\Psi_s$ changed in response to the formation of N$_2$O$_3$ and HNO$_3$ during generation of ozone from ambient air via electric discharge (Brown and Roberts 1988). However, we have previously found that foliar N concentration of seedlings and mature trees either decreased or did not change in response to elevated ozone or acid rain, respectively (Momen and Helms 1995).

Decreased $\Psi_s$ might be caused by an increased concentration of osmotically active metabolites in response to an ozone-induced increase in allocation of photosynthate to foliage at the expense of decreased root growth. Alteration of carbon partitioning by elevated ozone has been attributed to disturbed phloem loading or increased accumulation of starch and sugar in foliage (Hanson and Stewart 1970, McLaughlin et al. 1982, Okano et al. 1984, Cooley and Manning 1987, Küppers and Klumpp 1988, Gorissen and Van Veen 1988, Edwards 1991, Gorissen et al. 1991, Olszyk et al. 1991, Smeluders et al. 1995). However, in this study, the decreased $\Psi_s$ is unlikely to be the result of an ozone-induced increase in the allocation of photosynthate to foliage because only very small changes in foliar C concentration occurred in response to elevated ozone (Momen and Helms 1995). We hypothesize that $\Psi_s$ was de-
Increased by elevated ozone as a result of osmotic adjustment (Morgan 1984, Salisbury and Ross 1985). Concurrent increases in sugar concentrations and decreases in starch concentrations in response to elevated ozone have been observed in many studies (Barnes 1972, Kozioł 1984, Ito et al. 1985, Cooley and Manning 1987, Friend and Tomlinson 1992, Kelly et al. 1993, Smuleaders et al. 1995). In our study, osmotic adjustment in response to elevated ozone is indicated by decreases in \( \Sigma_p \) and TLP, and concurrent increases in \( \Sigma_p \) and \( V_t \) but unchanged \( V_s \). Because osmotic adjustment facilitates water extraction and turgor maintenance during drought (Hsiao et al. 1976, Tyree et al. 1978, Turner and Jones 1980, Tyree and Jarvis 1982, Morgan 1984, Turner 1986, Momen et al. 1992, Momen et al. 1994), our findings support the hypothesis that elevated ozone enhances drought resistance of plants (Temple 1990).

Ozone-induced osmotic adjustment and increases in \( \Sigma_p \) should enhance plant growth; however, many reports suggest that plant growth is inhibited by elevated ozone. Growth or plastic (irreversible) cell expansion depends partly on the rate at which \( \Sigma_p \) exceeds a threshold value that controls cell-wall extensibility (Salisbury and Ross 1985). Our finding that elevated ozone caused an increase in \( \varepsilon \) may indicate an increase in the threshold pressure required to permit cell expansion and hence a decrease in growth rate. Decreased elasticity of guard cell walls may also affect plant growth through changes in stomatal conductance (Runeckles and Rosen 1977) and water use efficiency. Therefore, the positive effects of osmotic adjustment and increased \( \Sigma_p \) on plant growth might be offset by the negative effect of decreased cell wall elasticity.

The interactive effects of acid rain and elevated ozone on \( \varepsilon \) and \( \Sigma_p \) could support the hypothesis (Heath 1980) that elevated ozone reacts with the plasmalemma and increases the permeability of membranes thereby enhancing the movement of S and N ions of acid rain into leaf cells leading to decreased \( \Sigma_p \). However, analysis of foliar chemistry (Momen and Helms 1995) indicated no increase in foliar N concentration of ponderosa pine seedlings or mature trees in response to increased rain acidity.

The effects of elevated ozone on water relations of ponderosa pine seedlings were similar to the effects of drought in many species (cf. Chapin 1991). It seems unlikely that elevated ozone induced water stress in seedlings as a result of decreased root growth and water uptake, because \( \Sigma_p \) of seedlings were not affected by twice-ambient ozone and were near maximum. Interactive effects of acid rain and elevated ozone indicate that the effects of one pollutant depended on the level of the other, and thus no accurate conclusion can be drawn from single pollutant studies in regions exposed to multiple pollutants. The large responses of seedling water relations to pollutants contrasted with those of mature trees and may indicate that the parent genotype had adapted to environmental stresses. It is possible that seedlings and mature trees differed in their responses to the treatments because seedlings were entirely exposed to pollutants whereas mature trees were not, or because the branches of mature trees were not entirely autonomous with regard to plant water relations. However, cellular damage by elevated ozone is local, and branches of mature trees have been found autonomous with respect to carbon transport (Cregg 1990, Mattson et al. 1990, Sprugel et al. 1991) and water relations (Zimmermann 1983, Tyree 1988).

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References


