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Effects of long-term ozone exposure and drought on the photosynthetic capacity of ponderosa pine (*Pinus ponderosa* Laws.)

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SUMMARY

Seedlings of ponderosa pine (*Pinus ponderosa* Laws.) were grown for three years under three atmospheric ozone concentrations – clean air (CF), ambient ozone (NF), and 1·5 times ambient ozone (NF150) – at a moderately-polluted site in the Sierra Nevada, under either well-watered or drought-stressed conditions. When the trees were 5 years old, photosynthetic capacities of 2-year-old, 1-year-old, and current-year needles were measured during August and September of the 3rd season of exposure. Current-year needles of NF150 trees had higher photosynthetic capacity than NF and CF trees during late summer, an effect due to greatly enhanced photosynthesis in well-watered plants that had lost older needles as a result of ozone damage. This photosynthetic compensation in well-watered NF150 seedlings was related to higher tissue nitrogen concentration in the current-year foliage and possibly to increased inorganic phosphate cycling, both responses to the loss of older needles. Drought-stressed NF150 seedlings were partially protected from ozone damage by decreased stomatal conductance and did not exhibit the same degree of photosynthetic compensation. No differences in photosynthetic rate were found between CF and NF seedlings or between well-watered and drought-stressed seedlings (across ozone treatments) in any needle age class.

Key words: Air pollution, ozone, *Pinus ponderosa* (ponderosa pine), photosynthetic capacity, drought stress.

INTRODUCTION

Air pollution, particularly ozone (O_3), has been implicated in damage to forest trees over widespread areas of the United States (McLaughlin, 1985). The effects of O_3 on foliar injury and growth reduction of trees in the montane forests of southern California are well documented (Miller *et al.*, 1989), and O_3 injury symptoms have been recorded on pines in the central and southern Sierra Nevada (Peterson *et al.*, 1987; Peterson, Arbaugh & Robinson, 1989; Duriscoe & Stolte, 1989). Among Sierran forest trees, ponderosa pine (*Pinus ponderosa* Laws.) and Jeffrey pine (*P. jeffreyi* Grev. & Balf.) are most susceptible to O_3, symptoms of injury including chlorotic mottle of needles and premature abscission of older foliage (Miller, Longbotham & Longbotham, 1983).

The physiological responses of trees to long-term exposure to O_3 are not well known. Ozone at ambient or higher concentrations has been shown to reduce photosynthesis in Fraser fir [*Abies fraseri* (Pursh) Poir.] (Tseng, Seiler & Chevone, 1988), eastern white pine (*Pinus strobus* L.) (Barnes, 1972; Reich & Amundson, 1985), loblolly pine (*P. taeda* L.) (Sasek & Richardson, 1989), and ponderosa pine (Miller *et al.*, 1969; Coyne & Bingham, 1981). However, no effect of O_3 on gas exchange was found in other studies on red spruce (*Picea rubens* Sarg.) (Kohut *et al.*, 1990), Scots pine (*Pinus sylvestris* L.) (Skärby, Troeng & Boström, 1987), or ponderosa pine seedlings (Bytnerowicz *et al.*, 1989). Increased net photosynthesis in current-year foliage of Norway spruce [*Picea abies* (L.) Karst.] exposed to ambient and elevated ozone was reported by Wallin, Skärby & Selldén (1990) and Eamus *et al.* (1990). Different durations of O_3 exposure and needle age classes used for measurement among these investigations makes interpretation of effects difficult.

In 1988 we began a project to expose ponderosa pine seedlings to combinations of air pollutant and natural stresses under controlled conditions near Kings Canyon National Park in the southern Sierra Nevada, California. Treatments included 3 levels of

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O_3, simulated precipitation at 3 pH values, 2 levels of dry deposition, and 2 irrigation regimes. During the first growing season only the drought treatment produced an effect on seedling growth. Visible symptoms of O_3 damage, consisting of necrosis or chlorotic mottle of older needles, were evident on 4% of the trees in the high O_3 treatment. In the second growing season both drought and elevated O_3 reduced growth, while precipitation chemistry had no effect. Drought decreased the negative impact of high O_3 concentration (Temple, Riechers & Miller, 1990).

At the end of the 2nd summer of our experiment, Hom (1990) measured the photosynthetic capacity of seedlings exposed to the O_3 treatments for one season. He found that current year needles of seedlings grown in elevated O_3 had higher rates of net photosynthesis than those exposed to clean air or ambient O_3 concentrations. He also found greater nitrogen concentrations in the needles of plants at high O_3 and suggested that this might be responsible for increased photosynthesis in response to stress. Needles from older age classes were not measured, however, and no conclusions could be drawn about whole plant gas exchange potential.

To corroborate these results, we measured photosynthetic capacity during the 3rd season of exposure, using seedlings that had been in the treatment chambers throughout the experiment. Our goals were to determine the photosynthetic potential of needles from 3 age classes (current-year, 1-year-old, and 2-year-old); to determine seasonal trends in photosynthetic capacity; and to relate gas exchange rate to tissue nutrient concentration. Light response curves and dark respiration were also measured but will be reported elsewhere (Hom & Riechers, 1991; Hom, unpublished data).

MATERIALS AND METHODS

Study site

The research site was located at Whitaker's Forest, a University of California forest preserve adjacent to Kings Canyon National Park and Sequoia National Forest at 1600 m elevation in the Sierra Nevada mountains (36° 37′ N, 118° 15′ W). Natural vegetation in the area is Sierran mixed conifer forest, characterized by giant sequoia [*Sequoiadendron giganteum* (Lindl.) Buchh.], ponderosa pine, sugar pine (*P. lambertiana* Dougl.), white fir (*Abies concolor* Gord. & Glend.), incense cedar [*Calocedrus decurrens* (Torr.) Florin], and California black oak (*Quercus kelloggii* Newb.). Thirty-six circular open-top chambers (Heagle, Body & Heck, 1973), 3·0 m in diameter and 3·5 m tall, were constructed in a clearing during June 1988; each had a dome top to exclude ambient precipitation. Thirty chambers were covered with transparent polyvinyl film and six others were left uncovered to serve as ambient air (AA) controls. Each covered chamber was connected to a blower which provided continuous air flow of approximately 105 m^3 min^{-1} (5 complete air changes min^{-1}). Blowers operated 24 h day^{-1} except during 28 September to 20 October 1988, when they were turned off at night (21.00 to 05.00 h).

Growth conditions

Covered chambers were randomly assigned to three O_3 treatments: charcoal-filtered (CF), non-charcoal filtered or ambient O_3 concentration (NF), and non-charcoal filtered plus O_3 added to achieve 1·5 times the ambient concentration (NF150). Chambers were maintained under these regimes from 6 July to 20 October 1988, 18 May to 2 November 1989, and 16 May to 2 November 1990. Ozone generated from dry, compressed air using a Griffin O_3 generator (Griffin Technics Corp., Lodi, NJ)[1], was added to the NF150 chambers as needed whenever blowers were operating (negligible HNO_3 was found to be added by this method). Full descriptions of the O_3 monitoring and control system, as well as acid precipitation and dry deposition treatments, can be found in Temple, Riechers & Miller (1992*a*). Ozone concentration in each chamber was monitored 5 times per hour and averaged to produce hourly means continuously during chamber operation.

Thirty-six 2-year-old ponderosa pine seedlings, planted in topsoil collected at the research site in pots 18 cm in diameter and 40 cm deep, were placed in each chamber on 17 June 1988. Pots were set into the ground inside liners to minimize temperature fluctuations in the root zone. Plants were watered with a drip system that provided 1·1 l of deionized water at each irrigation. Well-watered (WW) plants received water approximately every 2 weeks from 1 July to 31 October 1988 and weekly from 1 May to 31 October 1989 and 1990; drought-stressed (DS) trees were watered every 3–4 weeks in 1988 and every 2–3 weeks in 1989 and 1990. Weighing of pots indicated that WW plants used about 50% of available soil water between irrigations and DS trees used nearly all of it (Temple *et al.*, 1992*a*). No fertilizer was added during the experiment. Chamber sides and tops were removed for the winter and plants received ambient precipitation. Twelve trees from each chamber were harvested for biomass determination at the end of each of the three exposure periods.

Physiological measurements

Seedlings were selected for physiological measurements from the three levels of O_3 exposure and AA chambers under the least acidic simulated rainfall treatment (two chambers at each ozone level). Two

[1] Mention of a product does not imply endorsement by the University of California or the USDA Forest Service.

well-watered and two drought-stressed trees were chosen from each replicate chamber, for a total of four trees per ozone/irrigation combination. The same 32 seedlings were used for all measurements.

Gas exchange was measured with a LI-COR 6200 photosynthesis system (LI-COR, Lincoln, NE, USA), which recorded transpiration and stomatal conductance simultaneously with photosynthesis; rates of CO_2 assimilation and stomatal conductance were calculated using the equations of von Caemmerer and Farquhar (1981). A specially-built cuvette, small enough (60 cm^3) to make measurement of single fascicles possible, allowed temperature control and use under artificial light. We measured photosynthetic capacity (A_{max}) at 26 ± 1 °C under saturating light (approximately 1700 μmol m^{-2} s^{-1} PPFD at 400–700 nm), conditions which we and Hom (personal communication) determined in preliminary experiments would produce maximum photosynthetic rates in these seedlings. Light was provided by three GE spotlight bulbs filtered through 3 cm of water to reduce heat load. Ambient air was used in all measurements.

Seedlings were removed from the open-top chambers 1–3 h prior to measurement and placed under filtered shade; this allowed stomatal reopening to occur, if necessary, on hot afternoons. Trees were sampled 1–3 (WW) or 2–4 (DS) days after irrigation; no change in stomatal response was detected during these periods (DS trees took a full day after watering to recover from previously-experienced stress). All measurements took place between 10.00 and 16.00 h Pacific Daylight Time (PDT).

Three individual fascicles from the main stem were sampled in each of three age classes on each tree: 1990 (current-year), 1989 (1-year-old), and 1988 (2-year-old). Ponderosa pine produces only one flush of needles per year. Selected fascicles came from the upper regions of the 1988 and 1989 age classes and the lowest part of the 1990 flush. For measurement, a fascicle was positioned in the cuvette so that its three needles did not overlap. Total needle surface area was calculated from measured fascicle length and diameter, assuming that each needle represented one third of a cylinder, and photosynthesis was expressed on this basis. Measurements began in mid-August, after the 1990 needles had matured sufficiently to be used without damage. Drought-stressed trees were sampled one week, well-watered trees the next. Each tree was measured three times; mid-August, late August to early September, and mid- to late September 1990.

After the second set of measurements, fascicles were collected, dried, frozen and transported to Riverside, California, for tissue nutrient determinations. A known weight of dried, ground tissue was digested in H_2SO_4 and HCl; P and N concentrations were determined colorimetrically with a Technicon autoanalyzer, and Ca, Mg, and K were determined by atomic absorption spectrophotometry. Specific leaf weights were calculated [SLW = dry weight (g) per unit leaf area (m^2)]. Potential photosynthetic nitrogen use efficiency (PPNUE) and phosphorus use efficiency (PPPUE) were calculated by dividing photosynthetic rate (converted to weight basis) by tissue nutrient concentration (molar basis); this gives an estimate of instantaneous nutrient use (Field & Mooney, 1986).

Gas exchange, SLW, and nutrient results were evaluated by analysis of variance (ANOVA) and Tukey's HSD test for differences among means using SYSTAT (Wilkinson, 1988). Ozone treatment was analysed as the main effect using chamber means ($n = 2$) as blocks; irrigation regime was treated as a split-plot factor. Needle age classes were analyzed separately. Provision was made for unequal sample size in cases where some needles were missing in an age class. Relationships between gas exchange rate (second sampling period, average per tree by age class) and tissue nutrient concentration were examined using linear regression.

RESULTS

Ozone concentrations

Mean seasonal 12-hour (08.00–20.00 h PDT) O_3 concentrations at the site, measured outside the chambers, were 68 nl l^{-1} in 1988 (July–October), 58 nl l^{-1} in 1989 and 67 nl l^{-1} in 1990 (May–October). Due to high nighttime O_3 levels at this mountain site, 24-hour averages were only slightly lower: 63, 54, and 62 nl l^{-1} for 1988, 1989 and 1990, respectively. During 1990, when physiological measurements were conducted, the seasonal 12-hour O_3 averages for the CF, NF and NF150 chambers were 13, 62, and 95 nl l^{-1}, respectively. The 12-hour O_3 average in the NF150 treatment exceeded 100 nl l^{-1} during July and August in both 1989 and 1990. Total measured O_3 dose over the three exposure seasons averaged 47 μl $l^{-1} \times$ h in CF, 220 μl $l^{-1} \times$ h in NF, and 350 μl $l^{-1} \times$ h in NF150 chambers (Temple *et al.*, 1992*a*).

Growth response

Average biomass parameters from the 3rd harvest for the trees used to measure gas exchange are given in Table 1. The well-watered NF150 trees averaged 19·5 % less biomass than WW CF trees, but under drought stress biomass was reduced only 11 % in NF150 compared to CF trees (data were not analyzed statistically). Needle biomass was particularly affected by O_3 exposure: only one WW NF150 tree retained any 1988 needles at the end of the 1990 exposure season and just two had 1989 foliage. In contrast, among DS trees just one NF150 seedling had lost all 1988 and 1989 needles. However, 1990 needle dry weights were comparable in NF150 and

Table 1. *Mean biomass values from 3rd harvest for ponderosa pine seedlings used in photosynthetic capacity measurements in 1990. Treatment abbreviations: CF, charcoal filtered; NF, ambient ozone; NF150, 1·5 times ambient ozone; AA, open-air control*

	Dry weight (g)							
	1988 needles	1989 needles	1990 needles	All stems	All roots	Total weight	Root/ shoot	Percentage of CF total
Well-watered								
CF	5·35	7·24	7·31	18·40	32·59	70·90	0·85	—
NF	4·58	6·82	8·56	18·68	33·81	72·46	0·90	102·2
NF150	1·11	3·86	8·62	15·53	26·01	57·06	0·85	80·5
AA	2·64	8·22	11·25	23·53	29·82	75·46	0·65	106·4
Drought-stressed								
CF	3·94	5·95	6·30	14·35	25·72	56·13	0·84	—
NF	5·74	4·95	5·86	15·90	29·45	61·90	0·91	110·9
NF150	3·68	4·20	5·76	14·66	21·60	49·90	0·80	88·9
AA	3·44	5·26	6·66	17·94	24·56	56·90	0·74	101·4

Table 2. *Net photosynthesis at light saturation (A_{max}, μmol CO_2 m^{-2} s^{-1}) (± SE) of 3 age classes of ponderosa pine needles measured during mid-August 1990. Treatment abbreviations: CF, charcoal filtered; NF, ambient ozone; NF150, 1·5 times ambient ozone; AA, open-air control. Values are means of 3 fascicles on each of 4 trees. Reported ANOVA output omits AA treatment*

Watering regime	Age class	Ozone treatment			
		CF	NF	NF150	AA
Well-watered	1988	3·06 ± 0·16	3·30 ± 0·17	2·74 ± 0·60	3·37 ± 0·17
	1989	3·81 ± 0·15	3·72 ± 0·21	3·11 ± 0·18	4·42 ± 0·21
	1990	3·08 ± 0·17	2·89 ± 0·21	3·91 ± 0·31	3·81 ± 0·16
Drought-stressed	1988	3·60 ± 0·18	3·12 ± 0·11	2·46 ± 0·27	3·85 ± 0·22
	1989	4·34 ± 0·21	3·66 ± 0·14	3·02 ± 0·38	4·63 ± 0·16
	1990	3·66 ± 0·17	3·24 ± 0·15	3·77 ± 0·52	4·12 ± 0·10

ANOVA results (without AA):

		$P < F$		
Source	d.f.	1988	1989	1990
O_3	2	0·58	0·09	0·28
Irrigation	1	0·95	0·74	0·75
O_3 X Irrigation	2	0·79	0·69	0·92

CF trees. Average biomass of WW and DS trees from NF chambers and AA plots was similar to the CF treatments (Table 1).

Physiological responses

Each set (time period) of gas exchange measurements was analyzed separately. In all three sets, AA trees had greater gas exchange rates than NF trees (Table 2), indicating an influence of the chambers on light-saturated photosynthetic capacity. This may have been due to 3–5 °C higher midday air temperatures in the enclosed chambers (Riechers, unpublished data). To distinguish O_3 effects from the apparent chamber effect, statistical analyses were repeated omitting the AA treatment, and those results are reported.

Photosynthetic capacities (A_{max}) of three needle age classes under the different O_3 and irrigation treatments are shown for mid-August (Table 2) and late September (Table 3). Two-year-old needles (1988 age class) tended to have lower A_{max} than those from 1989 or 1990. Drought stress did not significantly affect A_{max} in any age class when considered across O_3 treatments. There were no differences due to O_3 treatment among 1988 needles, but analyses include only surviving needles: only two WW NF150 trees had any 1988 foliage by late September, and these needles had surprisingly high photosynthetic rates (Table 3). For the other WW NF150 trees, obviously, photosynthesis by the 1988 age class was zero. During mid-August the effect of O_3 on A_{max} of 1989 needles was nearly significant ($P = 0·09$), with NF150 trees having lower rates than

Table 3. *Net photosynthesis at light saturation (A_{max}, μmol CO_2 m^{-2} s^{-1}) (± SE) of 3 age classes of ponderosa pine needles measured during late September 1990. Treatment abbreviations are given in Table 2. Values are means of 3 fascicles on each of 4 trees, except where indicated.*

Watering regime	Age class	Ozone treatment CF	NF	NF150
Well-watered	1988	2·94 ± 0·12	2·73 ± 0·19	3·76 ± 0·28[a]
	1989	3·84 ± 0·21	4·11 ± 0·11	3·12 ± 0·27[b]
	1990	3·57 ± 0·20	3·64 ± 0·14	5·01 ± 0·42
Drought-stressed	1988	2·65 ± 0·12	2·93 ± 0·19	2·82 ± 0·18[c]
	1989	3·87 ± 0·15	3·69 ± 0·14	4·04 ± 0·11[c]
	1990	4·22 ± 0·14	3·71 ± 0·15	4·33 ± 0·20

ANOVA results (without AA):

Source	d.f.	$P < F$ 1988	1989	1990	
O_3	2	0·83	0·89	0·01	NF150 > NF = CF
Irrigation	1	0·14	0·87	0·98	
O_3 X Irrigation	2	0·15	0·93	0·42	

[a] Mean of three fascicles on one tree and one fascicle on another.
[b] Mean of three fascicles on each of two trees.
[c] Mean of three fascicles on each of three trees.

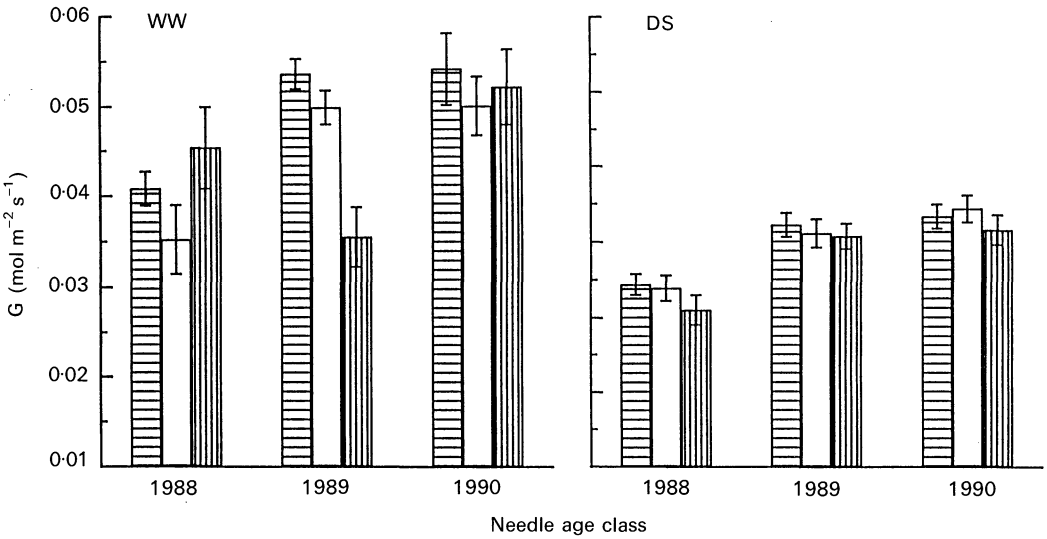

Figure 1. Needle conductance to water vapour (G) measured in late September 1990. Error bars represent one standard error of the mean. Treatment abbreviations: WW, well-watered; DS, drought-stressed; CF, charcoal filtered; NF, ambient ozone; NF150, 1·5 times ambient ozone. Within each age class, WW trees (ozone treatments combined) had significantly greater G than DS trees ($P < 0·01$). ▤, CF; □, NF; ▥, NF150.

NF or CF (Table 2). This effect was not apparent in the later measurements, in part because needles that were quite chlorotic in mid-August abscised by early September. There were no significant effects on A_{max} during the early September sampling period in any age class (data not shown). By late September, 1990 NF150 needles had significantly greater ($P = 0·01$) A_{max} than NF and CF needles (Table 3). The increase was due to greatly enhanced rates in WW trees, with a much smaller difference apparent in DS plants (Table 3).

Because transpiration rates of the ponderosa pine needles were very low, leaf conductance calculated using the LI-COR 6200 exhibited a great deal of variability. We regarded the conductance and transpiration measurements as being near the limits of the ability of the system. Drought-stressed trees had lower conductance than WW in 1989 needles ($P < 0·01$) during early September, with 1990 nearly significant ($P = 0·07$; data not shown). All three age classes were significantly affected by drought during late September ($P < 0·01$; Fig. 1). Ozone treatment had no detectable effect on conductance, and there were no significant interactions.

Exposure to O_3 had no effect on foliar concentrations of calcium, magnesium or potassium.

Table 4. *Nitrogen concentrations (percent dry weight) of ponderosa pine needles from three age classes collected in early September. Treatment abbreviations: WW, well watered; DS, drought-stressed; others as given in Table 2. Values followed by different letters in the BOTH row (WW and DS together, testing for ozone effect) and within an age class in the ALL column (O_3 levels together, testing for irrigation effect) are significantly different at $P < 0.05$. The ozone X irrigation term was not significant for any age class*

Age class	Water	CF	NF	NF150	ALL
1988	WW	0·683	0·663	0·591	0·656a
	DS	0·922	0·894	0·754	0·856b
1989	WW	0·670	0·716	0·820	0·735a
	DS	1·032	0·972	0·761	0·922b
1990	WW	0·626	0·650	0·883	0·720a
	DS	0·999	0·893	0·991	0·961b
1990	BOTH	0·813ab	0·772a	0·937b	—

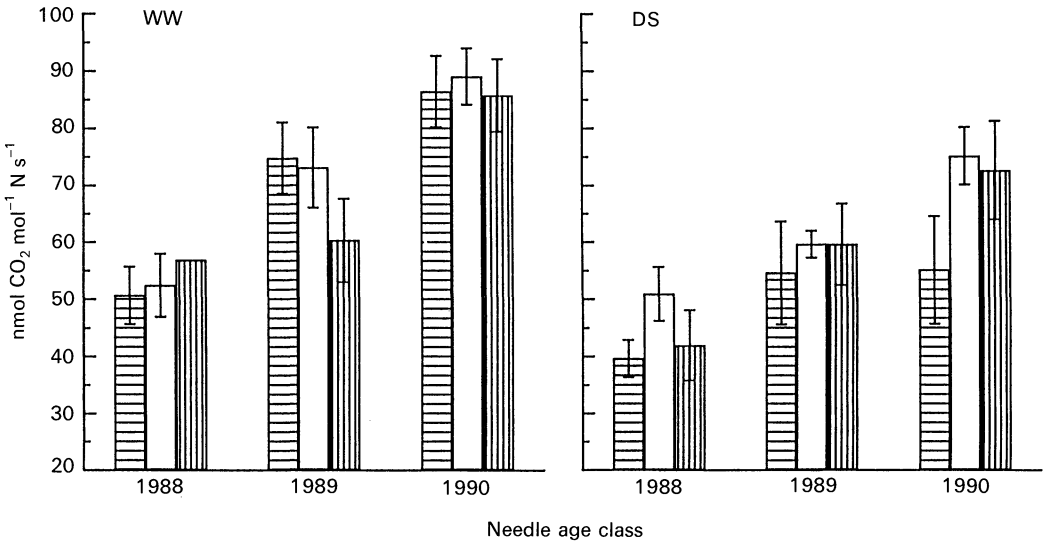

Figure 2. Potential photosynthetic nitrogen use efficiency (PPNUE) of ponderosa pine seedlings determined in early September 1990. Error bars represent one standard error of the mean. Treatment abbreviations: WW, well-watered; DS, drought-stressed; CF, charcoal filtered; NF, ambient ozone; NF150, 1·5 times ambient ozone. For key see legend to Figure 1.

Drought stress affected only potassium in 1989 needles (WW > DS, $P < 0.05$), with no ozone-drought interactions (data not shown). Tissue nitrogen concentration was significantly increased by drought in all needle age classes ($P < 0.05$; Table 4). Nitrogen concentration was also significantly greater in 1990 needles from NF150 relative to CF and NF trees ($P = 0.03$). The drought-O_3 interaction was on the borderline of significance ($P = 0.09$) for 1990 needles: N concentration in WW NF150 seedlings was 35 % greater than in NF or CF, but DS NF150 did not show an increase (Table 4). Phosphorus concentration was highest in 1990 needles, with DS significantly greater than WW ($P = 0.045$; data not shown). There were no other significant effects on P concentration. Specific leaf weight increased with needle age but was not affected by drought or O_3 (data not shown).

Potential photosynthetic nitrogen use efficiency (PPNUE) decreased with needle age (Fig. 2) and tended to be lower in DS than WW trees in all age classes ($P = 0.052$, 0·14, and 0·10 for 1988, 1989 and 1990 needles, respectively). Ozone treatment did not affect PPNUE. Potential photosynthetic phosphorus use efficiency (PPPUE) was not significantly altered by drought or O_3 (Fig. 3). Photosynthetic capacity was positively correlated with both N and P concentration in WW trees ($P < 0.01$), but regression coefficients for both nutrients were low ($R^2 = 0.30$ for N, $R^2 = 0.42$ for P). Relationship between A_{max} and foliar N and P levels in DS trees were not as strong ($P = 0.027$, $R^2 = 0.14$ for N, and $P = 0.056$, $R^2 = 0.11$ for P). Combining DS and WW trees produced significant regressions, but again coefficients were low ($P < 0.01$ for both, with $R^2 = 0.18$ for N and $R^2 = 0.24$ for P).

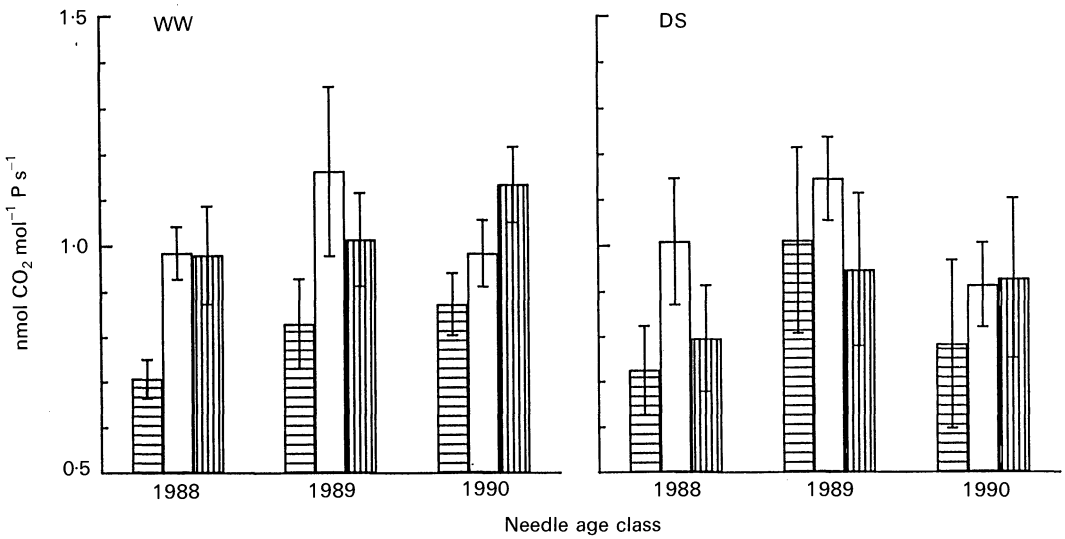

Figure 3. Potential photosynthetic phosphorus use efficiency (PPPUE) of ponderosa pine seedlings determined in early September 1990. Error bars represent one standard error of the mean. Treatment abbreviations: WW, well-watered; DS, drought-stressed; CF, charcoal filtered; NF, ambient ozone; NF150, 1·5 times ambient ozone. For key see legend to Figure 1.

DISCUSSION

Our results emphasize the importance of measuring gas exchange on more than one leaf age class in evergreen species such as ponderosa pine. We confirmed the finding of Hom (1990) that current year foliage of ponderosa pine seedlings exposed to above-ambient O_3 had enhanced photosynthetic capacity compared to CF and NF plants; in our study the increase was most significant in well-watered trees late in the growing season (Table 3). No significant changes in A_{max} were observed in NF relative to CF trees. We found little decrease in A_{max} of older needles under elevated O_3, despite the presence of chlorotic mottle on 1988 and 1989 needles in some NF150 seedlings (personal observation and data in Temple *et al.*, 1992*a*); however, yellowing did not exceed 50 % of leaf surface in needles used for measurement. Use of only the reasonably green needles in older cohorts probably underestimated the effect of ozone damage on photosynthetic potential. Abscission of ozone-damaged foliage on some NF150 plants in late August and early September resulted in zero photosynthetic contribution from those age classes.

One-year-old (1989) needles in NF and CF plants tended to have the highest photosynthetic rates early in the season, but with time the current year foliage become more important (see Tables 2, 3). This pattern is similar to that reported by Helms (1970) for ponderosa pine growing in the field. In contrast, in NF150 trees the 1990 needles always had higher rates. Coyne & Bingham (1982) also found that current year needles of ponderosa pine in the heavily polluted San Bernardino mountains had the highest photosynthesis rates, even in trees showing only slight visible O_3 injury. Increased photosynthesis by current year foliage seems to be a compensatory response to O_3 damage.

Drought-stressed plants had lower leaf conductance to water vapour than WW plants (Fig. 1), even under the conditions of plentiful soil moisture used in our measurements. This undoubtedly resulted in decreased O_3 uptake and explains the smaller growth reduction, relative to CF, seen in DS compared to WW trees (Table 1). In 1989, consistently lower stomatal conductance was measured in recently watered DS seedlings and only extremely low conductance was found during the second week after irrigation (P. Temple, unpublished data). Less foliar injury was recorded on DS trees throughout this study (Temple *et al.*, 1992*a*). Thus, although it reduced growth, drought stress partially protected seedlings from damage by decreasing total O_3 dose, an effect seen in other species as well (Tingey & Hogsett, 1985; Dobson, Taylor & Freer-Smith, 1990). Consequently, compensatory increases in photosynthetic rate were not as apparent under drought stress.

What physiological mechanism might account for higher photosynthetic capacity in 1990 foliage? Wallin *et al.* (1990) measured higher light-saturated photosynthesis in current-year needles of NF compared to CF seedlings of Norway spruce, but offered no real explanation for the finding. Stimulation of photosynthesis in ozone-fumigated Norway spruce was detected the spring after fumigations had ceased by Eamus *et al.* (1990) and was attributed to a decrease in winter hardening and accelerated spring de-hardening. In our experiment, the highest individual photosynthetic rates were measured in well-watered NF150 seedlings that had lost more or all older needles. A similar phenomenon was reported by Sasek *et al.* (1991) for loblolly pine exposed to

2·25 and 3·0 times ambient O_3. This response resembles that seen when plants are defoliated experimentally or by insects (Sweet & Wareing, 1966; Welter, 1989). Increased photosynthetic rate in defoliated plants may be due to changed nutrient relationships, changes in source-sink relations in the plant, or altered hormone concentrations (Welter, 1989). Hormonal regulation of photosynthesis is difficult to evaluate experimentally (Sharkey, 1985); we discuss the other two possibilities below.

Field & Mooney (1986) described a robust relationship between leaf nitrogen concentration and photosynthetic capacity across a range of species. In our study, the relationship between A_{max} and foliar N and P concentrations was strongest for well-watered plants. Drought-stressed trees had higher N concentrations than WW but did not exhibit higher A_{max}, resulting in lower PPNUE and suggesting that their lower stomatal conductance (Fig. 2) limited photosynthetic potential. Negative correlation between water use efficiency and nitrogen use efficiency was also noted by Field, Merino & Mooney (1983). High A_{max} in 1990 needles of WW NF150 trees was associated with high N concentration (Tables 3, 4). Examination of PPNUE in 1990 needles of WW trees [Fig. 2; PPNUE = 86·4, 89·1, and 85·8 nmol CO_2 $(mol^{-1}\ N)s^{-1}$ for CF, NF and NF150, respectively] suggests that 1990 needles in all treatments made maximum use of available nitrogen. Reallocation of N from prematurely abscised older needles to new tissue in ozone-damaged trees could have resulted in greater photosynthetic capacity in that tissue.

Von Caemmerer & Farquhar (1984) attributed increased photosynthetic capacity in bean plants after partial defoliation to an increase in both RuBP carboxylase (Rubisco) activity and RuBP-regeneration capacity. High N concentration in 1990 needles in our NF150 plants suggests an increase in Rubisco content and potential activity. RuBP regeneration depends on the products of the light reactions of photosynthesis and on triose phosphate utilization (Farquhar, von Caemmerer & Berry, 1980; Sharkey, 1985). High illumination used in our measurements makes it unlikely that light reactions *per se* limited RuBP regeneration. In many plants, however, the supply of inorganic phosphate (P_i) necessary for continued ATP production may be limited by the rate of sucrose synthesis (Sharkey, 1985). In WW NF150 plants, loss of older needles created greater relative demand for photosynthate from 1990 needles in roots and stems (needle weight : root + stem weight ratio was 0·39 in CF trees and only 0·33 in NF150 trees; Table 1). Greater demand could stimulate sucrose translocation (Wardlaw, 1990) and allow increased sucrose synthesis, releasing P_i. Examination of phosphorus use efficiency in 1990 needles of WW plants suggests that this may have occurred [Fig. 3; PPPUE = 1·134, 0·984 and 0·872 nmol CO_2 $(mol^{-1}\ P)s^{-1}$ for NF150, NF and CF trees, respectively]. Seedlings exposed to high O_3 tended to make more efficient use of available P. It appears that a combination of nitrogen reallocation and increased demand for photosynthate resulted in the observed increase in photosynthetic capacity in 1990 needles.

How well could greater photosynthetic capacity in current year foliage compensate for loss of older needles in ozone-injured ponderosa pine seedlings? For a rough estimate, we multiplied photosynthetic rates measured in late September by total foliage biomass present at the final harvest. Maximum potential photosynthetic yield was 740, 808 and 716 μmol CO_2 s^{-1} for well-watered CF, NF and NF150 trees, respectively (not significantly different between treatments). These estimates considerably exceed actual whole plant photosynthetic potential, as many needles are shaded by others, especially in older age classes, PPFD is not saturating for the entire day, and midday stomatal closure may occur. Nevertheless, this suggests that, under our experimental conditions at least, compensatory photosynthesis by new needles in trees exposed to high O_3 can reduce potential growth reductions caused by O_3 injury to older foliage. That compensation is not completely effective is evidenced by our growth results (Table 1, and Temple *et al.*, 1992*b*); this may be due to the cumulative effect of slightly lower total net assimilation and greater dark respiration rates in ozone-injured seedlings (Hom, unpublished data).

Extrapolation of results from chamber studies to plants in the field can be difficult because of inherent chamber effects on plant growth (Olszyk *et al.*, 1992). We found consistently higher light-saturated photosynthetic rates in open-grown (AA) versus chambered (NF) seedlings (Table 1), even though concurrent conductance measurements were similar and foliar nitrogen concentrations were the same (unpublished data, this study). Final average biomass of AA plants was about 14% greater than NF plants in both irrigation treatments [Temple *et al.*, 1992*b*; in contrast to our small subsample (Table 1)]. Although the dome tops over AA plots made midday light levels comparable in both settings, at low sun angles the AA plots may have received more light (polyvinyl film covering the chambers reduced ambient light less than 10% at midday, however). We attribute the photosynthetic capacity difference mainly to the consistent 3–5 °C elevation in midday temperatures recorded inside the chambers. For all our seedlings, we altered the soil moisture regime considerably from what would be found in the field, an unavoidable consequence of growing plants in pots. However, our trees were grown in unamended native soil, unlike those in many chamber experiments, and at the final harvest had tissue nutrient concentrations indistinguishable from similarly-aged seedlings growing in nearby forest clearings

(Temple and J. McBride, unpublished data). Thus despite the presence of some chamber effects, we feel that our results make possible useful predictions about ponderosa pine in its natural setting.

If continued urbanization and population growth in the Central Valley lead to greater O_3 levels, ponderosa pine seedling and tree growth in the Sierra will be affected to an even greater degree than seen at present (Peterson *et al.*, 1989). The ability of seedlings, and probably mature trees, to compensate photosynthetically for lost foliage may help reduce the impact, but because older trees have a much smaller proportion of their foliage in the youngest age class, compensation may be much less effective in mature trees (Amundson *et al.*, 1991). In California's Mediterranean-type climate, soil dries progressively through the summer (Barbour & Major, 1988). We measured the highest net photosynthetic rates in current year foliage late in the growing season, but under natural conditions trees affected by high O_3 during June and July, when soil moisture is usually available, would be unable to compensate with greater photosynthesis in a dry September. Thus plants growing in what should be mesic, productive sites could be most severely affected by increased O_3 levels. Experimentally-induced drought stress reduced the impact of high O_3 but also reduced growth and nutrient use efficiency. Seedlings and trees growing in particularly dry microsites may be protected to some degree from O_3 damage, but their growth will be limited by stomatal closure.

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