

O₃ uptake and drought stress effects on carbon acquisition of ponderosa pine in natural stands

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Summary

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- The effect of O₃ exposure or uptake on carbon acquisition (net assimilation (*A*) or gross photosynthesis (*P_g*)), with and without drought stress, is reported here in 40-yr-old-ponderosa pine (*Pinus ponderosa*) trees.
- Maximum daily gas exchange was measured monthly for 12 trees at four sites differing in pollutant exposure over two growing seasons with above- and below-average annual precipitation. Gas exchange measures were estimated between sampling periods using a generalized additive regression model.
- Both *A* and *P_g* generally declined with cumulative O₃ exposure or uptake at all sites. As a response variable, *P_g* was slightly more sensitive than *A* to cumulative O₃ exposure. As a metric, O₃ uptake vs exposure permitted slightly better statistical resolution of seasonal response between sites.
- The effect of late summer drought stress was statistically significant only at the moderate pollution site, and combined synergistically with O₃ exposure or uptake to reduce *P_g*. The general additive model allows the user to define a deleterious level of cumulative O₃ exposure or uptake, and to quantitatively assess biological response.

Key words: *Pinus ponderosa* (ponderosa pine), ozone (O₃) uptake, pollution effects, assimilation, photosynthesis, drought stress.

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Introduction

Ponderosa pine (*Pinus ponderosa*) is one of the most sensitive trees to oxidant air pollution in the western USA (Miller *et al.*, 1983). The long-term effects of oxidant exposure on this species have been well established from changes in basal area (Peterson *et al.*, 1991) and canopy health (Miller *et al.*, 1989). Ozone exposure indices for plants are based on hourly O₃ concentrations equal to or greater than a specified level, for a specified duration during the growing season (Stockwell *et al.*, 1997). Exposure to O₃ decreases whole plant carbon (C) gain, either through a decline in photosynthetic capacity (rubisco: Coyne & Bingham, 1982; Sasek & Richardson, 1989; Schweizer & Arndt, 1990, photosynthetic pigments: Tausz *et al.*, 2001), increased respiration (Amthor, 1994), or decreases in stomatal conductance (*g_s*, Weber *et al.*, 1993). Photosynthesis is a sensitive attribute that responds to within-growing season exposure to O₃, and is directly related to

biomass accumulation. For this reason, it was chosen as the biological response variable to O₃ in this study.

We used a general additive model to estimate whether O₃ exposure or O₃ uptake was a better metric. Net assimilation (*A*) was similar across three southern Californian sites that differed by 13% and 24% by total O₃ exposure over the growing season (Grulke, 1999), and by 28% and 51% by total O₃ uptake (calculated for the same trees used in this study, Grulke *et al.*, 2002). That paper describes higher O₃ uptake early in the summer at the moderately high pollution site, and higher O₃ uptake at the high pollution site in mid-summer. Was O₃ uptake a better metric than O₃ exposure in explaining biological response?

It was also possible that another measure of C acquisition was *A* more sensitive response variable than *A* to oxidant pollution. Foliar respiration increases with oxidant exposure (Amthor, 1994), but statistical significance is difficult to demonstrate because within site variability in tree response

increases with pollutant exposure (Grulke, 1999). There are greater differences between trees because O₃ sensitivity is expressed to a greater degree with increasing exposure (Coyne & Bingham, 1981). Growing season length and temperature differences modify foliar respiration (Landsberg, 1986), and thus *A* at our four sites. For this reason, we tested whether gross photosynthesis (*P_g*, assimilation plus daytime foliar respiration) was a more sensitive response variable than *A* to O₃ exposure.

Ponderosa pine-dominated coniferous forests commonly experience a period of summer drought where conductance to CO₂, water and O₃ is limited in late summer. In Mediterranean climates, even in years with above-average precipitation, *g_s* declines in this species after mid-July with declining soil water availability (Coyne & Bingham, 1982; Temple & Miller, 1998; Grulke & Retzlaff, 2001; Panek & Goldstein, 2001). Stomatal closure decreases oxidant uptake, but drought is also stressful and the combined effects may be deleterious (Heber *et al.*, 1995). Panek & Goldstein (2001) demonstrated a short-term protective effect of drought stress in unwatered vs watered plantation-grown ponderosa pine. In this paper, we tested whether drought stress was protective or deleterious to *P_g* at sites differing in pollutant exposure in natural stands of ponderosa pine.

Materials and Methods

Research sites

Four stands dominated by *Pinus ponderosa* Dougl. ex Laws. were chosen within the Sierran mixed conifer zone (*sensu* Barbour, 1988). Three of the stands, Barton Flats (BF), Strawberry Peak (SP) and Crestline (CR), had been exposed to significant pollution deposition for at least 50 yr in the San Bernardino Mountains (Miller & McBride, 1999), and the fourth, near Lassen Volcanic National Park (LS), had low oxidant exposure, near global background levels. The southern Californian stands were distributed across a pollution

gradient, from moderate O₃ exposure (BF, 250–258 ppm h, accumulated over the 6-month growing season, on a 24-h basis; Table 1), to moderately high (SP, 278–290 ppm h), to high O₃ exposure (CR, 341–346 ppm h). Nitrogen deposition ranged from 6 to 9 kg N ha⁻¹ yr⁻¹ at Barton Flats to 30–40 kg N ha⁻¹ yr⁻¹ at Crestline (Kiefer & Fenn, 1997). Lassen had low oxidant exposure (164–181 ppm h).

Field measurements were taken over three growing seasons differing in the degree of drought stress experienced. In 1993 and 1995, the total annual precipitation was average or above relative to a 118-yr regional record (Big Bear Dam, San Bernardino County Water District). In 1994, total annual precipitation was 20% below average. The 1993 summer was preceded by a year of above-average precipitation. In all three summers, less than 5% of the total annual precipitation was received between May 15 and October 1. In both average- and below-average precipitation years, the upper soil horizons dried to < 2% soil moisture by mid-July (Grulke, 1999), and water sources deep in weathered bedrock were probably utilized in late summer (Hubbert *et al.*, 2001). Higher soil moisture has been reported directly under mature tree canopies (Temple & Miller, 1998) perhaps attributable to hydraulic lift (Dawson, 1994), but this was not found for the 40-yr-old tree age class. In this study, predawn xylem potentials < -1.2 MPa were considered stressful (Grulke *et al.*, 1998 for the southern California sites; Grulke & Retzlaff, 2001 for Lassen; Table 1).

Representativeness of selected trees

At each site, a regionally typical multiage stand was chosen, and a plot was established to include at least 40 trees in the 20- to 60-yr-old tree age class. Trees in this broad tree age class have similar gas exchange, growth rates, phenological patterns, and physiological attributes (Grulke & Retzlaff, 2001). Twelve trees with average values relative to the population of this tree age class at each site were chosen for seasonal gas exchange measurements. Attributes used to

Site	Lassen	Barton Flats	Strawberry Peak	Crestline
Latitude, N:	40°20'11"	34°09'42"	34°14'00"	34°14'05"
Longitude, W:	121°35'04"	116°51'00"	117°08'12"	117°19'12"
Elevation, m	1700	1820	2240	1800
O ₃ , ppb h ^{ab}	38 [42]	62 [64]	76 [69]	80 [80]
Soil N, % ^c	0.01	0.08	0.11	0.20
Annual ppt (cm) ^d	115	90	96	98
Ψ _{PD} , MPae	-1.1 [-1.4]	-1.1 [-1.7]	-0.8 [-1.6]	-1.0 [-1.5]

Table 1 Site characteristics across the pollution gradient from the atmospherically clean site, Lassen, and from east to west across the San Bernardino Mountains east of Los Angeles, CA, USA

^aHourly O₃ concentrations were averaged 24 h per day, April 15 through October 15. ^bThe first value in each pair for the average precipitation year, and the second value in brackets is given for the below-average precipitation year. ^cFrom Grulke *et al.* (1998). ^dSan Bernardino County Water District e predawn xylem potential given is the lowest seasonal value obtained in early September for Lassen (Grulke & Retzlaff, 2001) and for the southern Californian sites (Grulke, 1999).

determine stand representativeness included: bole diameter, total tree height, percent chlorotic mottle of 1-yr-old needles, number of needle age classes retained, and the distance to the nearest conspecific tree. At Crestline and Strawberry Peak, sampled trees were widely separated and had little canopy or root interaction. Distribution at Barton Flats and Lassen was characterized by clumps of trees separated by open areas, typical of droughty environments.

Microenvironmental monitoring

Microenvironmental conditions of the sites have been summarized in Table 1. Variables were monitored every 5 min, and recorded hourly over the growing season in 1993, 1994 and 1995 on a data logger (Model 21x, Campbell Scientific, Logan UT, USA). Photosynthetically active radiation (PPFR, model 190 s, LiCor Instruments, Lincoln, NE, USA), needle and air temperature (copper-constantan thermocouples, 0.01 cm dia., Omega Engineering, Stamford, CT, USA), and O₃ concentration (model 1008, Dasibi Environmental Corporation, Glendale, CA) were measured. For O₃ concentrations, sample air was drawn from 3 m above the ground and 2 m from a building where instrumentation was housed. Air was passed through a Teflon filter to eliminate dust. Ozone monitors were calibrated monthly from May through October with a tertiary standard traceable to the National Institute of Standards and Technology. Calibration of the monitors passed auditing annually with a transfer standard certified by the US Environmental Protection Agency.

Gas exchange measures

Net assimilation rate (A) was measured using the standard methodology for A (closed photosynthetic system, Model 6200, LiCor Instruments, Lincoln, NE, USA). Maximum daily A was measured monthly on clear days, on 1-yr-old needles, on each of two primary branches, on 40-yr-old trees from the end of May to the end of September in 1993 and 1994. Gas exchange was measured between 0830 and 1330 h in May and June, and between 0830 and 1130 h in the remaining months at ambient CO₂, leaf temperature, vapor pressure deficit, and under saturating light conditions (> 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for at least 20 min before and during measurement). A 0.25-l cuvette was used to measure two fascicles per branch per tree. The flow and CO₂ zero was checked every 2 h and gain for the CO₂ infrared gas analyzer was checked daily with a tertiary calibration gas (+1 ppmv; cross-checked with a secondary standard traceable to the National Institute of Standards and Technology). Gas exchange was reported on a needle surface area basis, determined from a geometric model constructed of fascicle diameter (0.02 mm) and needle length (mm).

To calculate gross photosynthesis (P_g), foliar respiration was measured after A on the same foliage: an insulated dark

cloth with an outer surface of reflective material was wrapped around the cuvette which maintained temperature within 2°C. Respiration was measured approximately 12–15 min after measures of A on the same needles to minimize photorespiration. The equilibration time was empirically determined.

Estimation of daily O₃ uptake

Site-specific models of g_s were constructed from monthly, diurnal measures of six trees per site in 1995 (Grulke *et al.*, 2002), and were used to estimate hourly g_s over the growing seasons in 1993 and 1994. Fitted curves for the compiled tree diurnals were produced by the nonparametric scatterplot smoother ('loess', Cleveland & Devlin, 1988). The technique uses locally linear or quadratic regression to estimate a relationship between the predictor, x , and the response, y . The 90th percentile diurnal g_s response was estimated for times between sampling dates (and for times and dates in different years) from the nonparametric regression models, measured times of dawn and dusk, hourly PPFR, the site-specific response between g_s and PPFR on foggy or cloudy days, and predawn xylem potential as a measure of drought stress (Grulke *et al.*, 1998). O₃ uptake was calculated from hourly estimates of g_s , hourly O₃ concentrations measured at each site, and a constant for the difference in diffusivity between O₃ and H₂O (Laisk *et al.*, 1989).

Statistical methods

Values from individual trees were used as replication within a site. Statistical significance was reported at $P = 0.05$. Boxplot and scatterplot smoothers were used to display the data. Boxplots are effective for comparing distributions and central tendencies of data from different groups. Scatterplot smoothers (Cleveland, 1979) are effective for visualizing overall patterns or mean relationships between two variates in the presence of natural variation. A generalized additive regression model (Hastie & Tibshirani, 1990) was used to study the type and significance of relationships between various explanatory variables and the response of interest. The following model was used for studying the effects of covariates on C acquisition:

$$(\text{C acquisition attribute}) = \mu + \text{drought} + f_1(\text{cumulative O}_3 \text{ metric}) + \varepsilon \quad [\text{Eqn 1}]$$

where the C acquisition attribute was either daily maximum A or P_g at a given site and day of year; drought was the effect of a categorical variable indicating whether the trees were under drought stress (predawn xylem potential < -1.2 MPa) at the time the observation was made; f_1 , f_2 , f_3 are nonparametric transformations of the cumulative O₃ metric (O₃ exposure or O₃ uptake); and ε is an estimate of random error. Day of year was not included in the equation because of

colinearity with O_3 exposure. The technique uses a locally weighted polynomial smoothing routine (Cleveland & Devlin, 1988) to estimate the nonparametric transformations simultaneously with the other terms in the model. The model lets the data suggest the shape of the relationship between the explanatory variables and the response of interest within the context of holding other variables in the model constant. Goodness-of-fit of the final models were assessed by producing plots of observed vs estimated values.

Results

Descriptive statistics of C acquisition attributes

The values of P_g were plotted against site, nondrought (predawn xylem potential > -1.2 MPa) or droughty (predawn xylem potential < -1.2 MPa) conditions, day of year, and cumulative O_3 exposure over the growing season to describe the averages and ranges in the data in its entirety (Fig. 1). Relative to trees at the other sites, P_g was significantly greater at the cleanest site, Lassen (Fig. 1a). P_g did not differ significantly between the three southern Californian sites (Fig. 1a).

P_g was significantly lower for droughty vs nondroughty periods in field measurements (Fig. 1b). P_g decreased with both day of year (Fig. 1c) and cumulative O_3 exposure for field data (Fig. 1d). The exception to this pattern was in early May, when photosynthesis was still increasing to full capacity from winter dormancy. For this reason, the relationship between cumulative O_3 exposure and P_g was described mathematically only for the period after late May (140th day of the year). Cumulative O_3 exposures did not differ significantly between 1993 and 1994 at any site.

Field measurements vs model estimates of C acquisition attributes

To assess the overall goodness of fit of the model, we plotted the field measurements of A and P_g vs the fitted C acquisition attribute for the same day of year (Fig. 2a,b). The model appeared to give an adequate fit to the data, and the regressions for each site between observed and fitted C acquisition was highly significant ($P < 0.01$). The percentages of explained variation in A at the four sites were 65% (CR), 66% (SP), 69% (BF) and 75% (LS). The percentages of

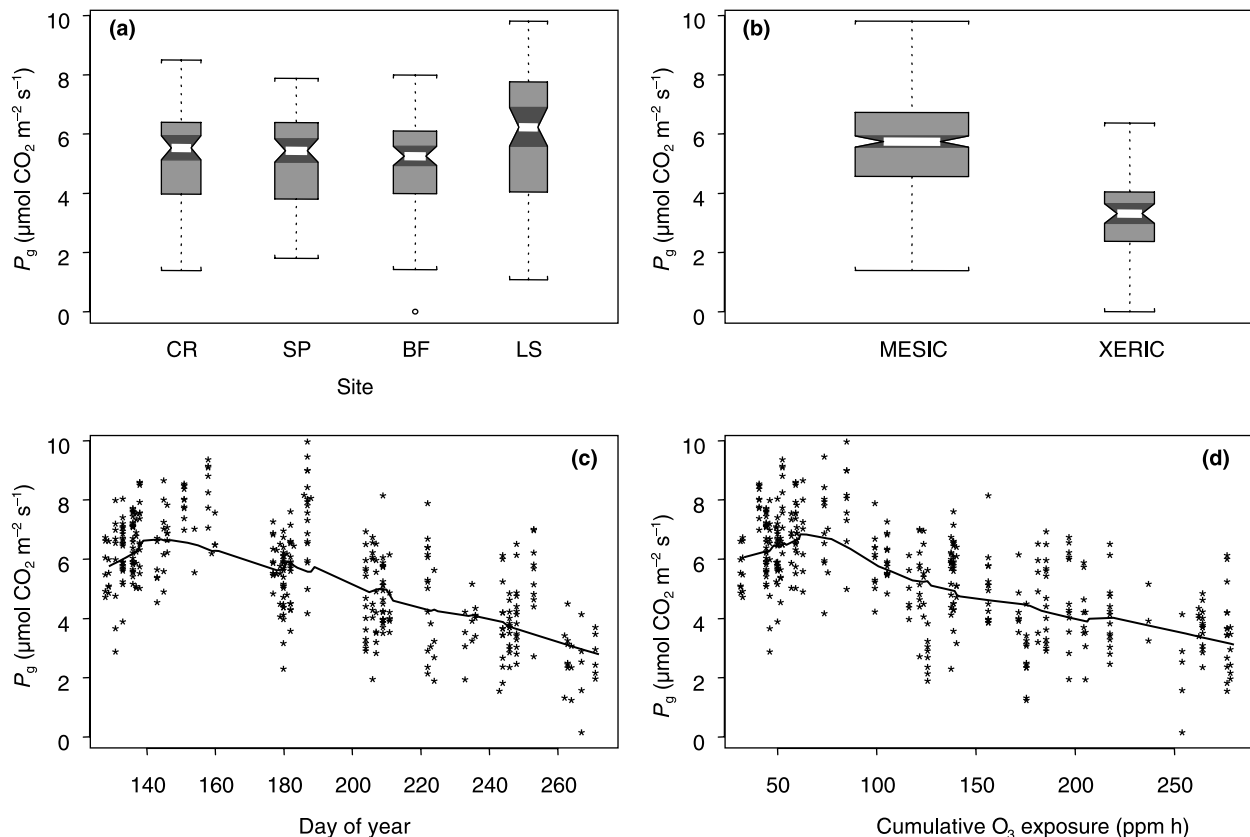


Fig. 1 Descriptive statistics for gross photosynthesis (P_g) for the entire data set. (A) by site, in order of highest to lowest pollutant exposure: CR, Crestline; SP, Strawberry Peak; BF, Barton Flats; and LS, Lassen Volcanic National Park. (b) For predrought (mesic, > -1.2 MPa ψ_{PD}) and drought-stressed periods (xeric < -1.2 MPa); (c) for day of year; and (d) for cumulative O_3 exposure. Widths of the bars are proportional to sample size. Median value and the 95% confidence interval are given as the center and notched regions of the boxes.

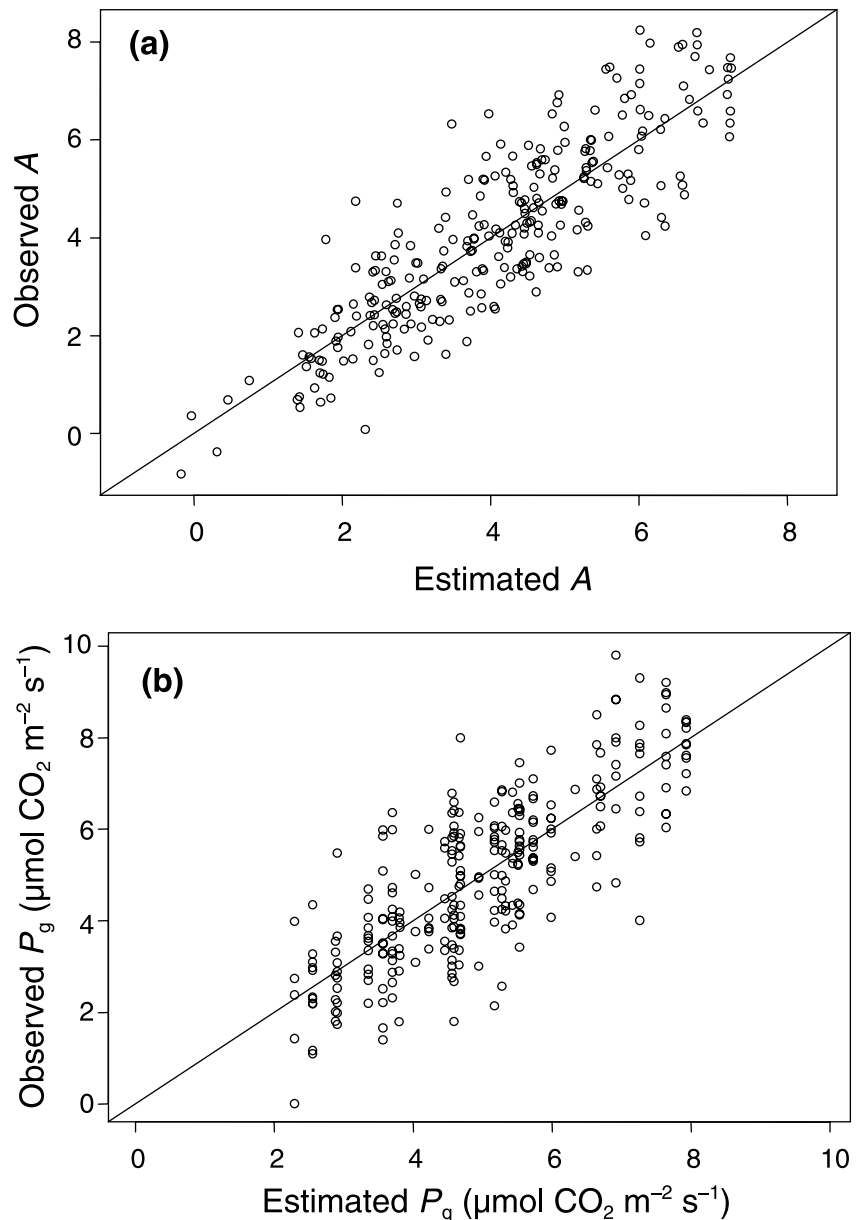


Fig. 2 (a) Relationship between observed and estimated (A) net assimilation rate (A) and (b) gross photosynthetic rate (P_g) for all sites. The data points are given relative to a 1 : 1 line.

explained variation in P_g at the four sites (in the same order) were 35%, 54%, 47%, and 74%.

Estimated effect of cumulative O_3 exposure on A

The general additive model allowed estimation of the effect of cumulative O_3 exposure, as cumulative O_3 exposure increased, on the biological response variable of interest (A) (Fig. 3). In this application, the zero line has been set to the population average value over the growing season at each site, but is user-defined. The effect of cumulative O_3 exposure on A was significant at all sites. Higher than average A was found at low O_3 exposure early in the growing season at all sites. At Crestline and Lassen, there was no further decline in A with

cumulative O_3 exposure in the remainder of the growing season. At the moderately high (Strawberry Peak) and moderate (Barton Flats) pollution sites, A declined linearly with cumulative O_3 exposure. The response line (the solid line in Fig. 3) crossed the population mean value of A at cumulative O_3 exposures of 200 ppm h at CR, 180 ppm h at SP, 175 ppm h at BF, and 120 ppm h at LS (Table 2). The 95% confidence intervals (the dotted line in Fig. 3) crossed the population mean values of the four sites at 130, 160, 150, and 100 ppm h, respectively. Although the predawn xylem potential did not differ significantly among sites in 1994 (Table 1), late summer drought stress was a significant variate on A at Barton Flats ($P < 0.001$). At all other sites, drought stress was not included in the final model because it

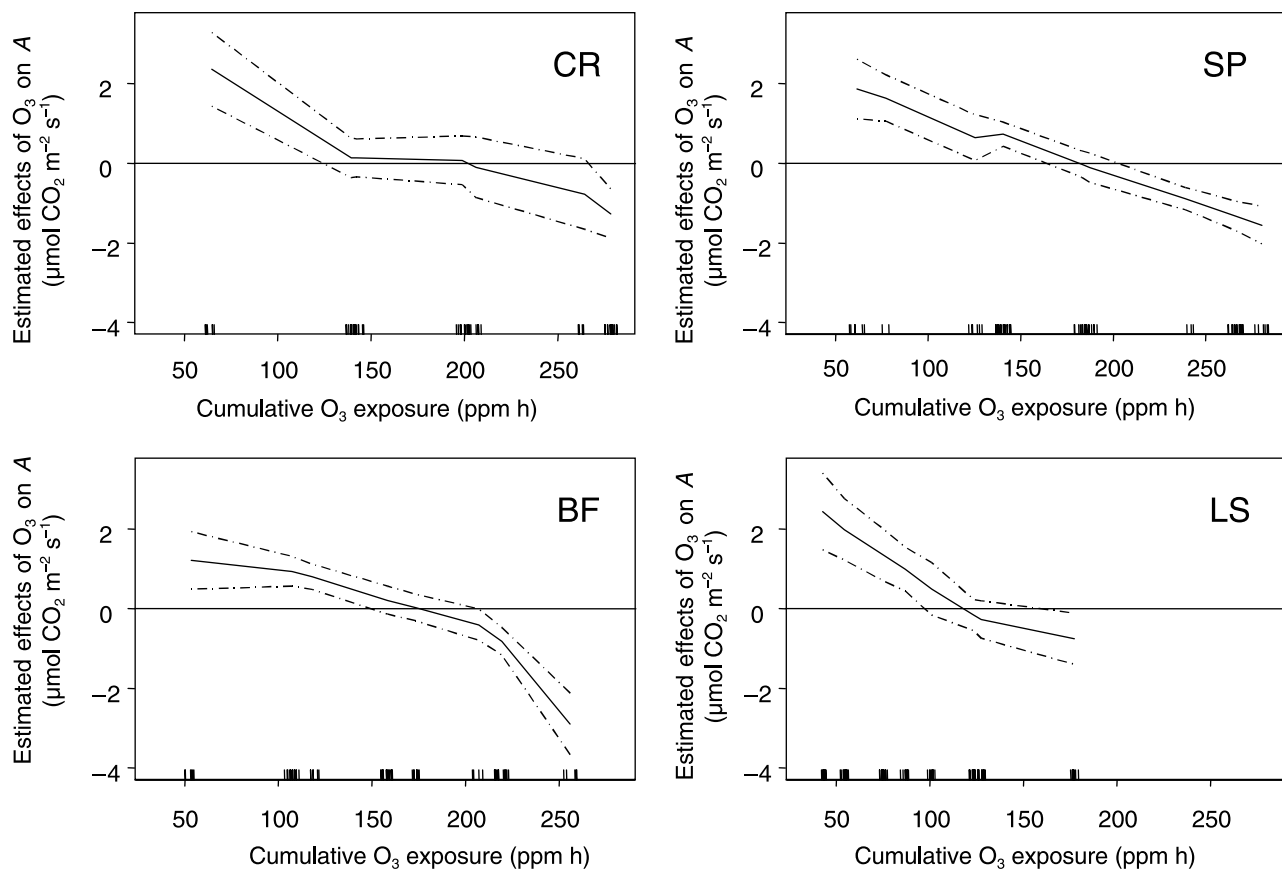


Fig. 3 Estimated effects of O_3 exposure on A with increasing cumulative O_3 exposure for the four sites. Small hatch marks on the x-axis indicate sample size. The solid line indicates the response line and the dotted lines indicate the 95% confidence interval. The zero line is set to the average value of A at each site. The point where the response line crosses the zero line indicates the point where the effects of O_3 elicit a lower value of A than the population average.

Table 2 Summary of the cumulative O_3 exposure (in ppm h) where the response line crosses the line indicating the population average for A (Fig. 3) and P_g (Fig. 4). The day of year (Date) corresponding to that cumulative O_3 exposure, for 90% maximum needle elongation growth, branch growth, and the onset of drought stress is also given in brackets for 1993 and 1994

Site	A , ppm h	Date	P_g , ppm h	Date	90% maximum Needle growth	90% maximum Branch growth	Onset of drought	Reference
CR	200	[209, 208]	180	[199, 198]	[225, 205]	[190, 180]	[> 205, > 215]	(1)
SP	180	[201, 207]	180	[201, 207]	[230, 220]	[190, 180]	[> 205, > 215]	(1)
BF	175	[220, 214]	160	[211, 204]	[235, 210]	[195, 185]	[> 205, n.d.]	(1)
LS	120	[250, 216]	115	[244, 211]	[225, 220]	[190, 160]	[270, > 205]	(2)

1, Grulke & Balduman (1999). 2, Grulke & Retzlaff (2001).

did not significantly modify the relationship between cumulative O_3 exposure and A .

Response of respiration to cumulative O_3 exposure

A stepwise regression was used to determine the role of leaf temperature, cumulative O_3 exposure, day of year, and drought stress on respiration. For the full data set, the inclusion of leaf temperature and day of year gave the best fit using the r^2 (adjusted for sample size) as well as Mallows

$C(p)$. Leaf temperature alone accounted for 45% of the variability, and day of year accounted for an additional 15% of the variability (Eqn. 1 in Table 3). If forced into the regression model, cumulative O_3 exposure accounted for only 0.5% of the variance. The order of variates had no effect on their inclusion into the equation.

Respiration was lower in late vs early summer, and lower in late summer of 1994 (20% below-average annual precipitation) than late summer 1993. Respiration was lowest of all at the site with the highest pollution exposure, probably due to

Table 3 Regressions for the relationship between daytime foliar respiration (RD, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and leaf temperature (TL, $^{\circ}\text{C}$), day of year (d), and cumulative O_3 exposure (ppm h), and predawn xylem potential (Ψ_{PD} , MPa). Early summer was defined as April 15 through July 19; late summer was defined as July 20 through October 15

Changes in within-season foliar respiration	
Whole growing season, late season 1992 through 1994 ($n = 684$)	
(1) $\text{RD} = -0.055 (\text{TL}) + 0.004 (\text{d})$	(Adj. $r^2 = 0.58$)
Early season respiration response	
(2) $\text{RD} = -0.062 (\text{TL}) + 0.003 (\text{cum } \text{O}_3)$	(Adj. $r^2 = 0.73$)
Late season respiration response	
(3) $\text{RD} = -0.046 (\text{TL}) - 0.002 (\text{cum } \text{O}_3) + 0.231 (\Psi_{\text{PD}})$	(Adj. $r^2 = 0.48$)

lower carbohydrate reserves (Grulke *et al.*, 2001). The cleanest site had greater respiration at a given temperature because growth was restricted to a shorter growing season and growth rates were greater on a daily basis (Grulke & Balduman, 1999). These factors suggested a separate analysis to determine the

effect of variates on early and late summer leaf respiration. In the early season response (late May to mid-July), both O_3 exposure and leaf temperature were significant variates in predicting foliar respiration. These variates combined accounted for 73% of the variation in leaf respiration (Eqn. 2 in Table 3). In late summer (post mid-July), leaf temperature, cumulative O_3 exposure, and predawn xylem potential were significant variates in predicting foliar respiration, and accounted for 48% of the variation in leaf respiration (Eqn. 3 in Table 3).

Estimated effect of cumulative O_3 exposure on P_g

The estimated effects of cumulative O_3 exposure on P_g were similar to that of A at two of the sites, Strawberry Peak and Lassen (Fig. 4). At Crestline, using P_g instead of A improved resolution of the response, and shifted the response line from 200 to 180 ppm h (Table 2). At the two most polluted sites, P_g declined linearly over the full range of cumulative O_3 exposure, and responses did not differ significantly between the two sites ($P = 0.66$) (Fig. 5). At Barton Flats, the estimated effects of cumulative O_3 exposure on P_g were similar to that on A except that the response line was shifted slightly from 175 to 160 ppm h,

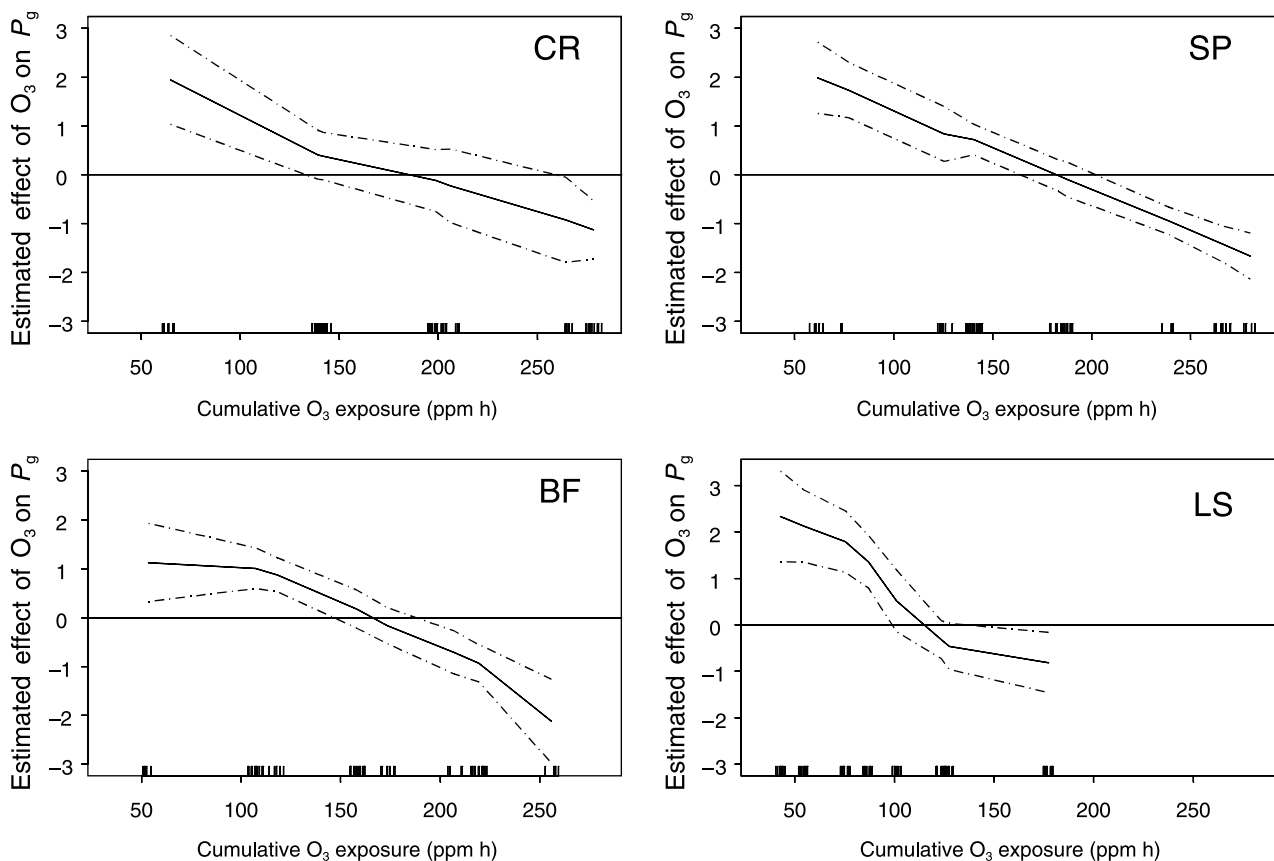


Fig. 4 Estimated effect of cumulative O_3 exposure on gross photosynthesis (P_g) for the four sites. Small hatch marks on the x-axis indicate sample size. The solid line indicates the response line and the dotted lines indicate the 95% confidence interval. The zero line is set to the average value of A at each site. The point where the response line crosses the zero line indicates the point where the effects of O_3 elicit a lower value of A than the population average.

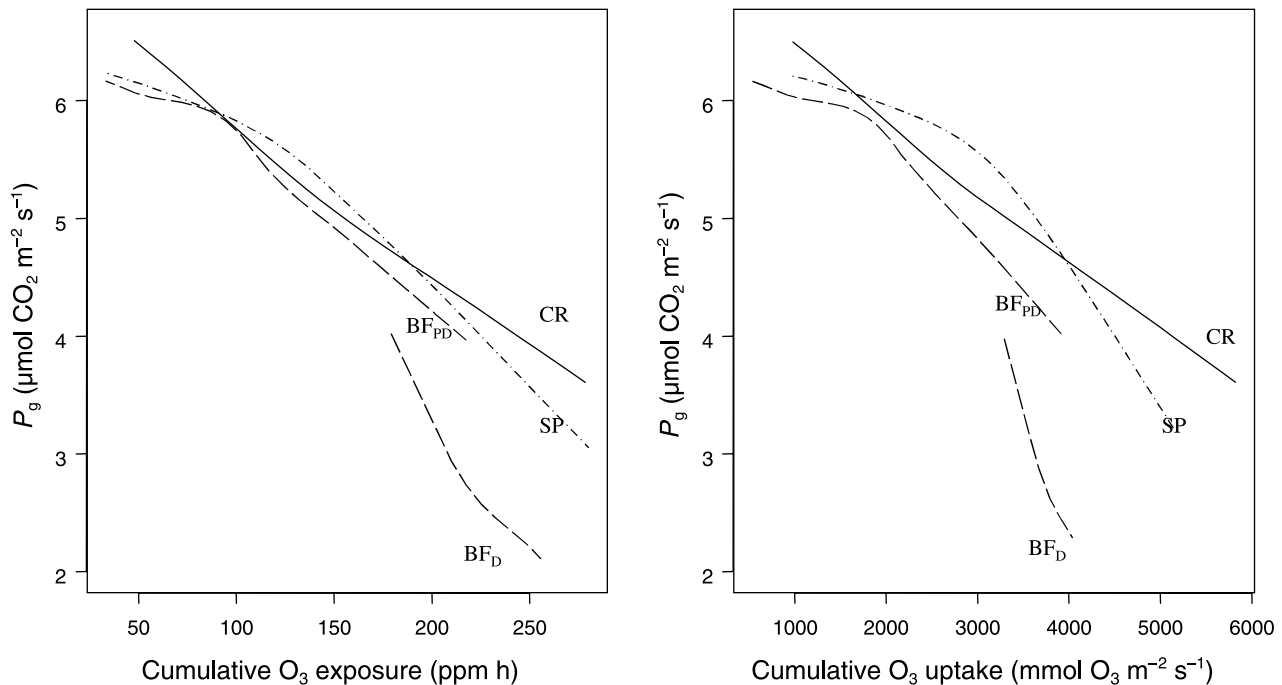


Fig. 5 Predicted seasonal course of maximum daily gross photosynthesis (P_g) vs cumulative O_3 exposure and uptake for the three southern Californian sites. The subscripts denote statistically significant predrought (PD) and drought-stressed (D) responses.

and the sharp decline at the end of summer was reduced due to increased respiration associated with drought stress (Eqn. 3 in Table 3). At Barton Flats, cumulative O_3 exposure < 100 ppm h had little effect on P_g . P_g declined linearly between 100 and 215 ppm h, similar to that of the two most polluted sites (CR, SP). By contrast, P_g declined linearly at Lassen at cumulative O_3 exposures less than 130 ppm h, but showed little further effect with increasing cumulative O_3 exposure. The response line and its 95% confidence interval (Fig. 4) crossed the population mean value of P_g at cumulative O_3 exposures of 180 and 130 ppm h at CR, 180 and 160 ppm h at SP, 160 and 150 ppm h at BF, and 115 and 100 ppm h at LS.

Similar to the analysis with A , predawn xylem potential was a significant variate on P_g only at Barton Flats in late summer ($P < 0.001$). Although the use of P_g corrected for the decline in A due to higher foliar respiration in late summer, the change in slope of the response line suggests that drought stress (predawn xylem potential < -1.2 MPa) combined with cumulative O_3 exposure to decrease P_g synergistically at this site. Although the percentages of explained variation at three of the sites were lower using P_g vs A alone, using P_g permitted better resolution of the whole growing season response at the most polluted site, and linearized the late summer response at Barton Flats.

Response of P_g to O_3 exposure vs uptake at southern Californian sites

The responses of P_g to cumulative O_3 exposure and uptake were estimated for the three southern California sites (Fig. 5).

Using A test for colinearity (Kleinbaum & Kupper, 1978), there was no significant difference in the response of P_g and cumulative O_3 exposure or uptake between the pre- and postdrought periods at Crestline or Strawberry Peak. Drought stress was a significant variate in the relationship between P_g and cumulative O_3 exposure ($P = 0.08$) and O_3 uptake ($P = 0.009$) only at Barton Flats, and this response been graphed separately. Under nondroughty conditions, the response of P_g to cumulative O_3 exposure did not differ among sites ($P = 0.66$), but the response to cumulative O_3 uptake differed significantly between Strawberry Peak and Barton Flats ($P = 0.03$). The response of trees at Crestline did not differ significantly from either of the other southern Californian sites, probably due to greater within-site variance in gas exchange.

Discussion

Ozone uptake may be limited in late summer due to lower g_s in response to phenological changes (Christmann *et al.*, 1999), O_3 exposure itself (Coynne & Bingham, 1982; Reich, 1987; Weber *et al.*, 1993), late summer drought stress (Christmann *et al.*, 1995), or a combination of these and other stressors. Nitrogen deposited with O_3 may further complicate response to oxidant stress. Crestline had twice the nitrogen content in the upper soil horizon relative to Strawberry Peak. In birch foliage, N-limitation increased, not decreased antioxidant concentrations (Polle, 1998).

The analyses presented here elucidated the effect of cumulative O_3 exposure or uptake on C acquisition. We estimated

effects of cumulative O_3 exposure on C acquisition, with respect to the population average of C acquisition. The estimated effects of O_3 were considered to be deleterious when the response line crossed the zero line: 50% of the population had gas exchange rates that were below average for that site (referred to subsequently as a 'threshold' response to cumulative O_3 exposure). The zero line could also have been defined at another value to assess cumulative and interactive effects of environmental stressors. For example in crops, the O_3 metric that would protect 50% of the crops from a 10% yield loss was used to illustrate the variability in response in different species (Tingey *et al.*, 1991). However, forest tree decline in response to cumulative O_3 exposure has not yet been so quantitatively defined. The general additive model is flexible, and allows the user to define which biological response variable is of interest and what level of decline from a site-specific maximum is deleterious. Also, thresholds can be compared between sites while the error in interpretation attributable to the underlying genetic structure or environmental conditions is minimized. Definition of a threshold response to cumulative O_3 exposure or uptake is important to parameterization of physiologically based models (e.g. Martin *et al.*, 2001).

The biological response attribute and the O_3 metric chosen can both influence the point where cumulative exposure results in deleterious effects. In this study of ponderosa pine, A and P_g declined with cumulative O_3 exposure, and P_g declined with cumulative O_3 uptake at all four sites. Using P_g instead of A as the biological response attribute improved resolution of the response at one site (Crestline, the most polluted) and improved interpretation of late season response at another (Barton Flats, the moderate pollution site). The cumulative O_3 exposure that elicited A deleterious effect varied little between the southern California sites, and differed little between A (200, 180, 175 ppm h, Crestline, Strawberry Peak, Barton Flats, respectively) and P_g (180, 180, 160 ppm h, respectively). The cumulative O_3 exposure that elicited a deleterious effect was considerably lower at the atmospherically clean site (120 ppm h for A or 115 ppm h for P_g). Perhaps long-term exposure has selected against the most sensitive individuals at the high pollution sites, and the trees chosen as representative of the site tolerated greater exposure before even the average C acquisition for the population of trees on the site was achieved. Precedence for this has been established for other conifers with long-term pollutant exposure (Oleksyn *et al.*, 1994; Prus-Glowacki *et al.*, 1999).

Gas exchange measures at Crestline had greater within-site variance than at the other sites, perhaps reducing the ability to detect statistical differences. The difference between sensitive and tolerant tree responses was marked (Coyne & Bingham, 1982; Grulke, 1999), and these differences would not have been evoked at sites with lower O_3 exposure. At high pollutant sites, more trees should be measured to reduce within population variance. With consideration for the error, cumulative O_3 exposure could have elicited a deleterious effect on C

acquisition as low as 130 ppm h for Crestline, and 160 ppm h for Strawberry Peak, 150 ppm h for Barton Flats, and 100 ppm h for Lassen.

The differences in threshold responses between some of the sites could be due to the exposure level itself. The statistical analyses presented here are not directly comparable with the pine responses to O_3 exposure compiled by Reich (1987). However, the two most polluted sites, Crestline and Strawberry Peak, experienced O_3 concentrations between his two lowest ranges (0.05–0.06 and 0.10–0.20 ppm). Reich (1987) describes a linear decline in A , with 10% reduction for the lowest range and 30% reduction for the second lowest range to a cumulative O_3 exposure of 120 ppm h. In our study, A also declined linearly at the two most polluted sites in the same exposure range, but A was reduced to a greater extent (by 60%) in our study. Tree response at the two least polluted sites described in our study were consistent with Reich (1987) from the point of view that the reductions in A with low O_3 concentrations and low doses were barely detectable in the field.

It could be argued that the threshold response to cumulative O_3 exposure was attributable to physiological changes associated with day of year, or the onset of drought stress. Day of year was not included in the generalized additive model because of collinearity with cumulative O_3 exposure. Although there was no statistically significant difference in O_3 exposure between the two years at any of the sites, the date when cumulative O_3 exposure became deleterious to C acquisition varied by 45 d among the four sites. The cessation of branch elongation growth, as a measure of phenology, was consistent across all sites (Table 3). Needle elongation growth was more variable. The best evidence that the decline in C acquisition was attributable to cumulative O_3 exposure is that the date corresponding to the threshold varied by 1 month between the 2 yr at the cleanest site, and predated the onset of drought stress in 1993. At that site, the date of cessation of needle elongation growth was similar in the 2 yr (220–225), and occurred *c.* 3 wk before the threshold in 1993 and *c.* 1 wk after the threshold in 1994. The threshold to cumulative O_3 exposure roughly corresponded to the onset of drought stress at the southern Californian sites, but predated the cessation of needle growth by 3–4 wk in 1993 and by 1 wk in 1994. Pre-dawn xylem potential differed on only one sampling date in the 3 yr of study across the San Bernardino Mountains (Grulke *et al.*, 1998). Among the southern Californian sites, the date when the threshold response was achieved varied by 2 wk.

Late summer drought stress reduced O_3 uptake at a ponderosa pine plantation with moderate O_3 exposure (Panek & Goldstein, 2001). Relative to the sites discussed here, their site has relatively high soil moisture availability through the growing season, and response might be most comparable with that presented for trees at Barton Flats in an average precipitation year. Lower g_s at Barton Flats may have afforded oxidant injury protection in early summer: there was a flat response of

P_g to accumulating O_3 exposure at values under 100 ppm h. In late summer, predawn xylem potential was a significant explanatory for C acquisition with cumulative O_3 exposure or uptake only at Barton Flats, a site with moderate O_3 exposure and low N deposition. The added effect of late summer drought stress appeared to be deleterious to A and P_g , not protective. In simulations of white fir response (*Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr.), A similar level of drought stress synergistically combined with moderate O_3 exposure to lower C gain (Retzlaff *et al.*, 1997). Drought stress was not a significant variate in the relationship between P_g and cumulative O_3 uptake at any other site, even in a drought year.

When evaluated on a site by site basis, the cumulative O_3 exposure that elicited A deleterious effect on C acquisition was similar across the southern California sites whether A or P_g was used as the biological response attribute. The use of P_g instead of A improved resolution of the response at the most polluted site, and helped to linearize the late season response at the moderate pollution site. The response of P_g to cumulative O_3 exposure or uptake also differed only slightly, but identified a different seasonal response in one of the three southern Californian sites (Strawberry Peak). High within-population variance due to greater environmental stress at the high pollution site prevented further resolution in response between sites. The use of a generalized additive model to isolate and estimate the effect of cumulative O_3 exposure was an effective quantitative tool. The response line and its error estimates the range of deleterious exposure to O_3 expected across the distribution of the west coast variety of ponderosa pine.

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