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Antioxidant Status of *Pinus jeffreyi* Needles from Mesic and Xeric Microsites in Early and Late Summer

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Summary

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The antioxidants ascorbate, glutathione, tocopherol, protective carotenoids, and water relation parameters were investigated in previous year's needles of *Pinus jeffreyi* growing in mesic and xeric microsites at Sequoia National Park, California, USA. Samples were taken before (June) and within (August) the summer drought period.

Total needle xylem water potentials (Ψ_L) varied little between trees in the different microsites in either June or August (pre-dawns ranging from -0.8 to -1.1 MPa and solar noon from -1.9 to -2.1). Maximum daily stomatal conductance was similar in the two microsites in June (55 mmol H_2O m^{-2} s^{-1}), but declined to a greater extent in xeric vs. mesic microsites by August (30 vs. 47 mmol m^{-2} s^{-1}). Predawn relative water contents showed a stronger seasonal decrease in needles from xeric microsites (132 to 110 water content as % dry weight) compared to mesic ones (134 to 120).

The sum of the photoprotective xanthophylls violaxanthin, antheraxanthin, and zeaxanthin increased from June to August (from 35 to 47 nmol μmol^{-1} chl^{-1}, P=0.015). The total glutathione contents decreased, and the ascorbate pool was in a more oxidized state in late summer. Significant differences between microsites were only detected for α-carotene contents which were lower in needles from xeric trees both in June and August. Total ascorbate pools were higher in needles from xeric trees, but the difference was not statistically significant.

The results demonstrate a seasonal change in antioxidant defense systems, but do not allow a clear evaluation of susceptibility to additional stresses (e.g. ozone) of xeric vs. mesic trees. A larger study is currently undertaken to clarify this question.

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Introduction

Drought stress induced stomatal closure limits the uptake of atmospheric pollutants and could alleviate air pollution effects on plants. On the other hand, both drought stress (SMIRNOFF 1993) and ozone induce the formation of toxic reactive oxygen species (ROS), and combined, may act synergistically. Antioxidant and photoprotective defense systems protect leaf tissues from adverse effects of ROS (POLLE 1997) and are known to be affected by drought stress (TAUSZ & al. 2001). In the level II approach of the critical loads concept adopted by the UN-ECE (FUHRER & al. 1997), water supply is believed to be an important factor modifying potential pollution responses. Presently, the interaction between ozone uptake, drought stress, and drought-induced formation of ROS, is not clear.

Both the ozone uptake and the detoxification potential of the foliage is relevant to understanding plant response, and to determining an effective physiologically active dose (WIESER & al. 2002). Pollution effects can also differ in drought-adapted or –stressed plants as a result of modifications to the antioxidant defense systems themselves. In a Mediterranean climate, the trees are exposed to regular summer drought periods, but the duration and severity of drought stress is governed by the water available to the root systems. Microsite conditions determine these water reserves. We investigated the antioxidant defense status of Jeffrey pine trees growing in mesic and xeric sites. The role of site differences on responses to atmospheric pollutants is discussed.

Material and Methods

The study site was a pine-dominated, Sierran mixed conifer forest (*sensu* BARBOUR 1988) on a south-facing slope, at 2170 m, in Sequoia National Park, California. Jeffrey pine trees (*Pinus jeffreyi* GREV. & BALF.) were identified as mesic or xeric by topographical position in the landscape, and on basal area growth patterns (ability or inability to respond to years of known average or above total annual precipitation, GRULKE & al., unpublished).

Diurnal stomatal conductance (g_s) was measured in pre-drought (June 24 to July 2) and during seasonal drought (August 18 to 27) on previous year's foliage. Foliage was measured on three branches per tree using both an open (model 6400, LiCor Instr., Lincoln, NE) and closed photosynthetic system (model 6200; using a modified technique to optimize for measurement of water vapor exchange, GRULKE & al. 2002). Stomatal conductance measured with the two instruments was similar at low values (< 3-4 mmol H_2O m^{-2} s^{-1}) and indistinguishable at values > 20 mmol H_2O m^{-2} s^{-1}. A geometric model of needle surface area was applied to measurements of fascicle diameter and needle length. Mid-canopy was accessed by a portable aluminum scaffolding reaching 15 m (Genie, Inc., Portland, OR).

Needle xylem water potentials (Ψ_L) were determined on previous years' fascicles at predawn and solar noon with a pressure chamber (PMS Inc., Corvallis, USA; PALLARDY & al. 1991). Relative water content (RWC) of the same needles was also determined (([field weight – dry weight]/[dry weight])*100 %).

Samples were taken at the end of June (before the summer drought) and at the end of August (after two months of summer drought). Fascicles of the previous year's flush were removed from sun-exposed, south-facing branches at the lower third of the canopy and immersed in liquid nitrogen immediately. The samples were lyophilized and sealed in plastic bags. Lyophilized needles

were ground in a dismembrator, and the needle powder was stored frozen in humidity proof plastic vials before it was subjected to HPLC analysis.

Pigments were determined on acetone extracts of the needle dry powder according to the HPLC gradient method of PFEIFHOFER 1989. Tocopherols were measured according to WILDI & LÜTZ 1996. Determination of ascorbate and dehydroascorbate was done by an isocratic HPLC method according to TAUSZ & al. 1996a and glutathione in its oxidized and reduced state was measured according to KRANNER & GRILL 1993. The concentrations of thylakoid located systems (carotenoids and tocopherol) were based per unit chlorophyll to relate their defense capacity to the light absorbance.

Statistical evaluations were completed using Statistica (StatSoft, USA, 1994). Figures show medians and median deviations (SACHS 1992, p. 336-337). Comparisons between sampling dates (June versus August) and microsites (mesic versus xeric) were evaluated using a two-way ANOVA approach with a repeat measures factor. Significant differences were verified by non-parametric methods (Wilcoxon test and Mann-Whitney test).

Results

Needle xylem water potentials (Ψ_L) differed little between microsites or within season. At solar noon, Ψ_L was about 1 MPa more negative than pre-dawn (Table 1). Maximum stomatal conductance decreased from June to August at both microsites, but this decrease was more pronounced in xeric trees (Table 1). A corresponding result was found for pre-dawn relative water contents (RWC), but not for the RWC at solar noon (Table 1).

Table 1. Water relations in previous year's needles of *P. jeffreyi* growing in xeric (X) and mesic (M) microsites in Sequoia National Park. Means ± SD of n=8 individual trees per microsite. P-values indicate significance level for two-way ANOVA analysis. RWC = relative water content [water in % of needle dry weight], Ψ_L = needle xylem water potential, g_s = stomatal conductance. x = interaction effect season x microsite, ns = not significant (P>0.05).

				ANOVA effects		
	micro-site	June	August	micro-site	season	x
predawn Ψ_L	M	-0.8 ± 0.1	-1.0 ± 0.2	ns	ns	ns
[MPa]	X	-1.1 ± 0.1	-0.9 ± 0.2			
solar noon Ψ_L	M	-1.9 ± 0.2	-2.1 ± 0.2	ns	ns	ns
[MPa]	X	-2.0 ± 0.3	-2.1 ± 0.4			
maximum daily g_s	M	55 ± 3	47 ± 5	ns	<0.001	0.015
[mmol H_2O m^{-2} s^{-1}*]*	X	56 ± 4	30 ± 5			
pre-dawn RWC [%]	M	134 ± 9	120 ± 7	<0.05	<0.05	ns
	X	132 ± 11	112 ± 6			
solar noon RWC	M	140 ± 8	105 ± 8	ns	<0.05	ns
[%]	X	126 ± 12	104 ± 5			

Most chloroplast pigment concentrations remained largely unaffected by the seasonal transition and by microsite differences. However, α-carotene was con-

sistently lower in xeric trees at both sampling periods. Also, the xanthophyll cycle pool was significantly larger in needles of xeric trees in August. No significant microsite or season effect was found for α-tocopherol concentrations (Table 2).

Table 2. Carotenoids and α-tocopherol [nmol μmol^{-1} total chlorophyll^{-1}], total chlorophylls, and the de-epoxidation state of xanthophylls in previous year's needles of *P. jeffreyi* growing in xeric (X) and mesic (M) microsites in Sequoia National Park. Means ± SD of n=8 individual trees are given for each microsite. P-values indicate significance level for two-way ANOVA analysis. V = violaxanthin, A = antheraxanthin, Z = zeaxanthin, DEPS = De-epoxidation state = (Z+0.5*A)/(V+A+Z)*100. dw = needle dry weight. ns = not significant (P>0.05).

				ANOVA effects		
	micro-site	June	August	micro-site	season	x
Neoxanthin	M	56 ± 5	61 ± 9	ns	ns	ns
	X	58 ± 10	58 ± 6			
Lutein	M	150 ± 14	176 ± 43	ns	ns	ns
	X	146 ± 18	151 ± 18			
V+A+Z	M	35 ± 8	47 ± 12	ns	0.015	ns
	X	42 ± 5	44 ± 5			
α-Carotene	M	25 ± 3	27 ± 10	0.040	ns	ns
	X	19 ± 5	22 ± 5			
β-Carotene	M	82 ± 12	94 ± 31	ns	ns	ns
	X	87 ± 16	85 ± 18			
α-Tocopherol	M	369 ± 67	455 ± 216	ns	ns	ns
	X	435 ± 80	399 ± 95			
De-epoxidation state [%]	M	56 ± 12	61 ± 9	ns	ns	ns
	X	48 ± 11	53 ± 8			
Total chlorophyll [μmol g^{-1}dw]	M	1.67 ± 0.23	1.53 ± 0.58	ns	ns	ns
	X	1.64 ± 0.32	1.70 ± 0.38			

The glutathione and ascorbate systems changed significantly from June to August, resulting in a smaller total glutathione pool in August (Fig. 1), and a more oxidized ascorbate pool (Fig. 2). Although trees from the xeric microsite had higher ascorbate concentrations, this trend was not statistically significant (Fig. 2).

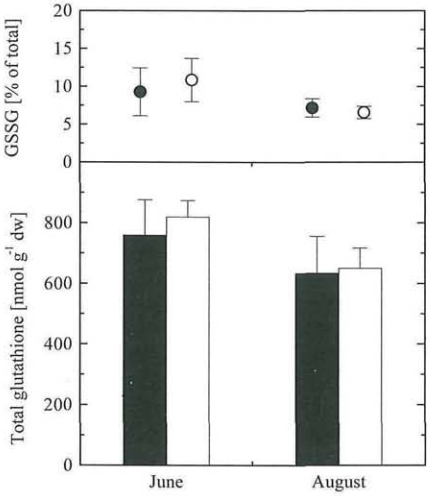

Fig. 1. The glutathione system in previous year's needles of *P. jeffreyi* growing at mesic (closed bars) and xeric (open bars) microsites in Sequoia National Park. Two-way ANOVA results: P=0.004 for the seasonal change (June versus August) in the total glutathione concentrations. All other effects were not significant (P>0.05). Bar height and error represent mean and S.D. of 8 trees in each microsite. dw = needle dry weight, GSSG = oxidized glutathione.

Discussion

Trees in xeric microsites limit stomatal water loss. Consequently, potential pollution uptake via the stomata is expected to be lower for trees in xeric microsites (GRULKE & al. unpublished data). Responses to seasonal drought were detected in the xanthophyll cycle system, which provides flexible protection from high light stress. In Mediterranean climates, afternoon high insolation is often concurrent with higher leaf temperatures and low vapor pressure deficits. Under these conditions, stomatal closure would lead to energy absorption in chloroplasts that would not be used in the dark reaction due to low substomatal concentrations of CO_2. The de-epoxidation of violaxanthin to zeaxanthin contributes to a harmless energy dissipation in the pigment bed in form of heat (MÜLLER & al. 2001). In the drought season, the pine needles contained a larger xanthophyll pool indicating a greater protection potential. On the other hand, the slight decrease of the glutathione pool and the higher oxidation state of the ascorbate pool in late summer suggest changes in the ascorbate-glutathione cycle, possibly with an increased turnover attributable to ROS scavenging activities. These observations also suggest a higher pressure on photoprotective and antioxidant systems in late summer in both microsites.

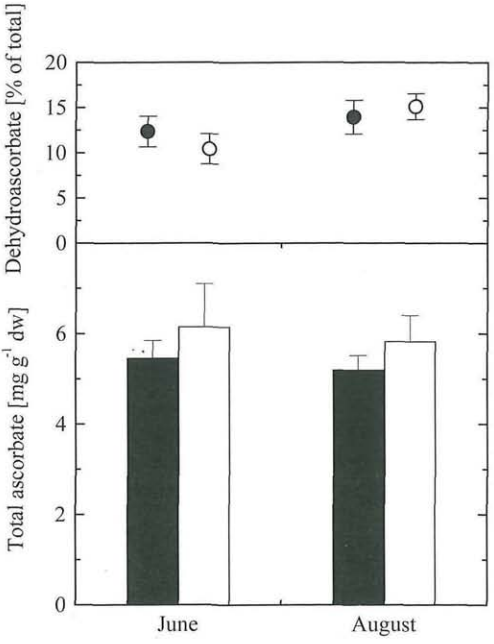

Fig. 2. The ascorbate system in previous year's needles of *P. jeffreyi* growing in mesic (closed bars) and xeric (open bars) microsites in Sequoia National Park. Two-way ANOVA results: P=0.005 for the seasonal change (June versus August) in the percentage of dehydroascorbate. All other effects were not significant (P>0.05). Bar height and error represent mean and S.D. of 8 trees in each microsite. dw = needle dry weight.

Total ascorbate contents were higher for xeric trees (although not statistically significant), and were present before the onset of seasonal drought. Lower α-carotene contents in xeric trees also suggest stronger oxidative conditions in the chloroplasts. This carotene is easily oxidized and often degraded under stress conditions (SIEFERMANN-HARMS 1994, TAUSZ & al. 1996b). With this small sample size (n=8 per microsite), few differences in the antioxidant defense systems between trees in the two microsites were significant. The trends observed here are strengthened in a larger study in progress (n=30 per microsite) that also details within-canopy variability in mesic and xeric microsites.

This study did not allow a definite evaluation of the potential for differing antioxidant defense capacities of mesic and xeric trees. However, permanent changes in the total ascorbate contents, and overall greater stress is suggested by the lower α-carotene contents in xeric trees, despite lower ozone uptake.

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