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Pigments and Photoprotection in Needles of *Pinus ponderosa* Trees with and without Symptoms of Ozone Injury

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Summary

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The best documented ozone-induced damages on forest trees are found on *Pinus ponderosa* Dougl. ex P. & C. Laws. in the San Bernardino Mountains in Southern California. The foliar injury to this species is characterized by specific visual symptoms ('chlorotic mottling').

In October 1997, ponderosa pine needles were collected at a severely impacted plot in the San Bernardino Mountains where individuals showing severe visual injury symptoms (symptomatic) and asymptomatic trees grow together. The symptomatic trees exhibit defoliation and thinned crowns caused by the premature loss of symptomatic needles (with typical 'chlorotic mottling') in late summer. At sampling time, the current year‘s flush and one-year old needles used for biochemical analysis did not show any or had only minimal symptoms of chlorotic mottle. In these needles, pigment concentrations in dark and light adapted needles were equal for the both symptomatic and asymptomatic trees, except for chlorophyll a decline in the previous year's needles of symtpomatic trees in the light.

However, the de-epoxidation state of the xanthophyll cycle in sun adapted needles of the symptomatic trees (35% de-epoxidised groups) was significantly higher than in needles of asymptomatic trees (20%). In the dark adapted stage, about 10% of de-epoxides were retained in all samples.

These results indicate an increased need for photoprotection of the symptomatic trees in the light and an early stage of (reversible) chlorophyll degradation in the light. Since these

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alterations are detectable before visual symptoms appear on the needles, they might be useful for early indication of risk associated with phytotoxic ozone stress to pines.

Introduction

In the San Bernardino National Forest in Southern California, the adverse effects of air pollution on the ecologically prominent forest species *Pinus ponderosa* Dougl. ex P. & C. Laws. is well documented (MILLER & al. 1998). The appearance of visible symptoms include 'chlorotic mottle' of the needles and accelerated needle abscission. Such symptoms are used for the monitoring of ozone injury of pine trees at field plots (MILLER & al. 1996).

In the San Bernardino Mountains trees showing severe symptoms (canopy thinning and chlorotic mottle on older needles) may grow in close neighborhood of individuals without such symptoms (MILLER & al. 1996). Younger needles of symptomatic trees may still be without visible symptoms, hence they may be used for the search for early indicators of injury.

Damage on plant organs must be preceded by changes at the cellular and biochemical level (WILD & SCHMITT 1995). Stressful conditions promote the formation of destructive oxygen species by the leakage of light driven electrons from the photosynthetic electron transport chain. These compounds may initiate chlorophyll destruction, eventually leading to visible chlorosis (ELSTNER & OSSWALD 1994). Since these reactions are driven by light energy, photoprotection in the thylakoids is the first line of biochemical defense against damaging reactions (FOYER 1997).

The present study compares photosynthetic pigments of the needles of symptomatic and asymptomatic trees in pursuit of the search for an early biochemical indication for ozone injury on *P. ponderosa*.

Materials and Methods

Sampling sites: The study was conducted in the San Bernardino Mountains (Southern California, USA) at the Dogwood plot.

Collection and preparation of material: Sun-exposed branches of dominant *P. ponderosa* were cut in the field at a height of about 6-10 m from 11 a. m. to 2 p. m. on cloudless days (October 1997). Some fascicles were removed immediately, needles cut and immersed in liquid nitrogen within seconds ('light-exposed needles'), the remaining branches were kept well watered, at room temperature (20 °C) and dark overnight. Fascicles were taken from the branches the next day in the laboratory (while protected against direct light) and frozen in liquid nitrogen ('dark-exposed'). The needle material was lyophilized and sealed in plastic bags. Lyophilized needles were ground in a dismembrator, the needle powder was stored frozen in humidity proof plastic vials before it was subjected to HPLC analysis.

Pigment analysis: Pigments were determined on acetone extracts of the needle dry powder according to the HPLC gradient method of PFEIFHOFER 1989.

Statistics: Statistical evaluations were completed using Statistica (StatSoft, USA, 1994) software package. Figures show medians and median deviations which are most suitable for small sample sizes (SACHS 1992). Comparisons between light adapted and dark adapted samples were calculated using the Wilcoxon matched pairs test, differences between symptomatic and asymptomatic trees by Mann-Whitney two sample test (SACHS 1992).

Results

Chlorophyll a and b concentrations were generally higher in previous year's needles than in current year's ones. In dark-exposed needles no difference in chlorophyll contents between symptomatic and asymptomatic trees was found. Light-exposed previous year's foliage of symptomatic trees exhibited significantly lower chlorophyll a concentrations, and also a tendency toward lower chlorophyll b contents, than the corresponding needles of symptomatic trees. In current year's needles, no significant differences were found among those groups (Table 1).

Table 1. Chlorophyll concentrations of *P. ponderosa* needles in mg g^{-1} DW. Medians ± median deviations of 5 individual trees. P-values indicate significance level for differences between asymptomatic (Asy) and symptomatic (Sym) trees, P>0.05 not indicated. D = dark exposed, L = light exposed samples.

		Previous year's needles		Current year's needles	
		D	L	D	L
Chl a	Asy	1.89 ± 0.25	1.97 ± 0.10	1.45 ± 0.22	1.33 ± 0.10
	Sym	1.91 ± 0.24	1.72 ± 0.11	1.43 ± 0.07	1.46 ± 0.30
			P=0.047		
Chl b	Asy	0.63 ± 0.07	0.62 ± 0.04	0.46 ± 0.07	0.39 ± 0.01
	Sym	0.62 ± 0.07	0.54 ± 0.04	0.45 ± 0.06	0.45 ± 0.12

On a total chlorophyll basis, differences between needle age classes in carotenoid contents were very small. Concentrations of the light harvesting xanthophylls lutein and neoxanthin, as well as in the carotenes (α- and β-carotene) did not differ significantly neither between light- and dark-exposed needles nor between symptomatic and asymptomatic trees (Table 2).

The behavior of the xanthophyll cycle was different between symptomatic and asymptomatic trees. The total pool size of the xanthophyll cycle pigments (violaxanthin, antheraxanthin, and zeaxanthin) amounted to between 25 and 32 µg mg^{-1} chlorophyll in all investigated needles. While all needle samples responded to darkening with violaxanthin formation at the expense of zeaxanthin, the extent to which these transitions amounted was different: the de-epoxidation status of the xanthophyll cycle (calculated as the ratio of de-epoxidised epoxide groups over total epoxide groups) was similar in all darkened needles, but it was significantly higher in previous year's light-exposed needles of symptomatic trees than in the corresponding needles of asymptomatic trees (P=0.028). In current year's needles, a trend toward higher zeaxanthin retention in the dark was observed, but due to high variations of the data it did not prove significant (Fig. 1).

Table 2. Carotenoids of *P. ponderosa* needles in µg mg^{-1} total chlorophyll. Medians ± median deviations of n=5 individual trees. Significant differences ($P<0.05$) between asymptomatic (Asy) and symptomatic (Sym) trees or between light (L) and dark (D) exposed state were not observed.

		Previous year's needles		Current year's needles	
		D	L	D	L
Neoxanthin	Asy	31 ± 1	34 ± 2	34 ± 1	32 ± 2
	Sym	33 ± 3	34 ± 2	34 ± 2	32 ± 3
Lutein	Asy	84 ± 2	83 ± 4	92 ± 2	84 ± 7
	Sym	87 ± 5	91 ± 5	93 ± 10	88 ± 6
α-Carotene	Asy	19 ± 1	18 ± 2	17 ± 3	16 ± 2
	Sym	15 ± 3	15 ± 1	15 ± 2	14 ± 2
β-Carotene	Asy	37 ± 2	35 ± 2	44 ± 2	43 ± 6
	Sym	42 ± 3	42 ± 3	42 ± 4	40 ± 3

Discussion

At sampling time, no or only mild visible injury was present on the investigated needles. Hence, the observed biochemical changes can be regarded as initial stages of the well documented chlorotic mottle.

The present results support the hypothesis that the light driven energy conversions of the photosynthesis are the processes where initial cellular damage occurs. In comparison to other conifer species CO_2-assimilation of *P. ponderosa* was most adversely affected by ozone impact (GRULKE & al. 1998), pointing to the particular susceptibility of the photosynthesis of this species. In the present study, with exception of a slightly higher zeaxanthin retention in young needles, all biochemical changes in the foliage of symptomatic trees were still reversible in the dark, but indicated an increased cellular stress-level in the light: higher de-epoxidation state of the xanthophyll cycle implies a higher dissipation rate of light energy and a lower light use efficiency of photosynthesis (DEMMIG-ADAMS & ADAMS 1994). This response reflects an increased need for photoprotection, probably due to impairments in photosynthesis (FOYER 1997). However, this protection was not sufficient to prevent a significant, but temporary loss of chlorophyll a in the light. Repair processes in absence of photostress might account for the recovery of chlorophyll a contents (RÜDIGER & SCHOCH 1988). Significant changes in pigments were only found in previous year's needles, but not in current year's foliage. In coincidence with the symptomology of ozone damage to *P. ponderosa* (older needles would develop visible injury and would be shed eventually), experimental reports show that chlorophyll destruction in *P. ponderosa* needles required at least two years of exposure to elevated ozone concentrations (TAKEMOTO & al. 1997). The development of the chlorotic mottle is the consequence of microscopical damages to photosynthetic active mesophyll cells (MILLER & al. 1996), and, cellular damages initiate in biochemical alterations in the photosynthetic apparatus (ELSTNER & OSSWALD 1994, WILD & SCHMITT 1995).

These pigment changes in the light are not the cause of injury, but an early response to impairments of the photosynthesis and, consequently, to the impact of light stress (FOYER 1997). Physiologically spoken, they demonstrate the involvement of photo-stress in the development of ponderosa pine ozone injury symptoms, and they might be used as an early indication of ozone phytotoxic effect.

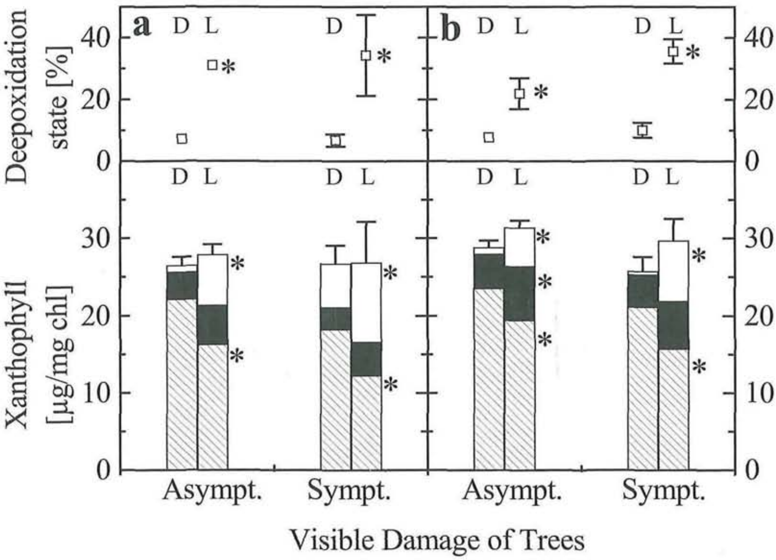

Fig. 1. The xanthophyll cycle pigments in needles of *Pinus ponderosa* trees at the Dogwood plot. a) Current year's needles, b) previous year's needles. Hatched columns = violaxanthin, closed columns = antheraxanthin, open columns = zeaxanthin. Columns and symbols show medians of 5 individual trees, error bars show median deviations of the pool size (not shown if within the symbol). D = dark exposed state, L = light exposed. Significant differences between light and dark exposed state are indicated by an asterisk.

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