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Stress-Physiological Response Patterns in Spruce Needles Relate to Site Factors in a Mountain Forest

By

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Summary

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Spruce trees are exposed to a complex of environmental factors at natural stands. The patterns of physiological plant responses allow conclusions regarding the stress combinations. At particular field sites small scale site factors, e. g. edaphic conditions, contribute to the stress level of trees together with large scale environmental factors such as altitudinal gradients.

In the present paper, physiological response patterns of spruce trees (*Picea abies* [L.] Karst.) are related to large scale and small scale environmental factors.

A principal component solution containing six accumulated components. Component scores were calculated by a two-way analyis of variance (ANOVA) with the large scale factor "altitude" and the small scale factor "water supply", in order to evaluate differences between sites and plant responses. Statistical trends for an altitude effect on scores were observed for antioxidants and pigment ratios (chlorophyll a/b and chlorophylls/carotenoids). For the effect of the site type (good or deficient water supply) significant correlations between water deficiency and antioxidant concentrations could be found. An independent support for this result was provided by measurements of osmotic potentials in saturated needles, indicating that enhanced contents of ascorbate were correlated to more negative osmotic potentials. This statistical approach showed that smale scale edaphic factors (e.g. deficient water supply) decisively contribute to the stress level of spruce trees.

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Introduction

Physiological stress responses reflect the situation of trees relevant to the present situation at field sites. The antioxidative defence system of plant cells is essential to withstand environmental challenges, since oxidative reactions are involved in most stress situations (ELSTNER & OSSWALD 1994). Responses of the antioxidants ascorbate and glutathione were found upon air pollution impacts, but also upon numerous other environmental factors (POLLE & RENNENBERG 1994). Changes in photosynthetic pigment composition reflect changes in plant vitality (LICHTENTHALER 1993). In field studies, such physiological responses are complex reactions to complex combinations of environmental factors, like climate, site factors, air pollutants etc. Response patterns underlying the variability of single physiological variables can be revealed by factor analytic techniques (TAUSZ & al. 1998).

In the present paper, physiological responses of spruce trees are related to large scale and small scale environmental factors. As large scale factors may be regarded altitudinal gradients, as the combination of high irradiation, harsher climate, and high ozone levels impose an increased stress level on the trees in high altitudes. Small scale differences refer to the site factors, such as nutrition and water supply which plays an important role in particular at limestone sites. The study aims at contributing to an interdisciplinary risk assessment for forest systems.

Materials and Methods

Sampling sites: Six sampled sites were situated in the Loisachtal (Tyrol, 47° 20' N, 10° 58' E) from 910 to 1500 m a.s.l. Sites were characterized according to plant communities based on OBERDORFER 1992-93. Sites with good water supply ("+") and temporarily dry sites ("-") could be distinguished (according to ELLENBERG & al. 1991).

Preparation of needle material: Branches of 23 dominant *P. abies* trees were cut in the field in late August 1993 and 1994 and kept cool and dark overnight. Needles were cut from the branches the next day in the laboratory, frozen in liquid nitrogen and lyophilized afterwards. Lyophilized needles were ground in a dismembrator, the needle powder was stored frozen in humidity proof plastic vials before it was subjected to biochemical analysis.

Biochemical analysis: Pigments were determined according to PFEIFHOFER 1989, ascorbic acid according to TAUSZ & al. 1996, and water extractable thiols according to GRILL & ESTERBAUER 1973. Peroxidase activity was measured spectrophotometrically according to KELLER & SCHWAGER 1971.

For the measurements of osmotic needle potentials, twigs were saturated with water and needles were removed afterwards and frozen. Determination was performed by means of an osmometer on needle material of the investigation year 1994.

Statistics: Statistical evaluations were completed using Statistica (StatSoft, USA, 1994) software package. Analyses in this paper were based on a data set of 230 sample trees of different field studies in Austria. This data set was subjected to a principal component analysis (TAUSZ & al. 1998). Original input variables were chloroplast pigments (neoxanthin, lutein, violaxanthin+antheraxanthin+zeaxanthin, chlorophyll a, chlorophyll b, α-carotene, β-carotene), pigment ratios (chlorophyll a/b, chlorophylls/carotenoids, xanthophylls/carotenoids, α/β-carotene), ascorbic acid, low molecular weight thiols, and peroxidase activities, in current year's and 1 year old needles. Principal component solution was accepted when eigenvalues were greater than 1 (Kaiser criterion), but the number of components was reduced according to the Scree plot criterion. Component scores were computed following variance-maximizing axes rotation and normalization (for a detailed description of the statistical procedure see TAUSZ & al. 1998). This analysis resulted in 6 physiologically meaningful components (Table 1). In the following these components are named according to the main contributing variables: Component 1 = pigment concentrations, component 2 = redox status of carotenoids, component 3 = chlorophyll a/b, component 4 = antioxidant concentrations, component 5 = chlorophylls/carotenoids, and component 6 = peroxidase activity.

Table 1. Principal component solution on 28 original variables for a collective of 230 individually sampled spruce trees from field sites in Austria (TAUSZ & al. 1998). Component loadings may be interpreted like correlation coefficients - high absolute values indicate a strong contribution of the original variable to the component. Positive loadings >0.50 (+), negative loadings < -0.50 (-), absolute loadings <0.50 (0).

(c = current year's needles, 1y = one year old needles, V+A+Z = violaxanthin+anteraxanthin+zeaxanthin, chl/ctde = chlorophylls/carotenoids, Xan/Car = xanthophylls/carotenoids, Chl a/b = chlorophylla/chlorophyll b, Carα/β = α-carotene/β-carotene)

		Component number					
Variable		1	2	3	4	5	6
Neoxanthin	c	+	0	0	0	0	0
	1y	+	0	0	0	0	0
Lutein	c	+	0	0	0	0	0
	1y	+	0	0	0	0	0
V+A+Z	c	0	0	+	0	0	0
	1y	+	0	0	0	0	0
Chl a	c	+	0	+	0	0	0
	1y	+	0	0	0	0	0
Chl b	c	+	0	0	0	0	0
	1y	+	0	0	0	0	0
α-Carotene	c	0	0	0	0	0	0
	1y	+	0	0	0	0	0
β-Carotene	c	+	0	0	0	0	0
	1y	+	0	0	0	0	0

		Component number					
Variable		1	2	3	4	5	6
Xan/Car	c	0	-	0	0	0	0
	1y	0	-	0	0	0	0
Chl/Ctde	c	0	0	0	0	+	0
	1y	0	0	0	0	+	0
Chl a/b	c	0	0	+	0	0	0
	1y	0	0	+	0	0	0
Car α/β	c	0	+	0	0	0	0
	1y	0	+	0	0	0	0
Ascorbate	c	0	0	0	+	0	0
	1y	0	0	0	+	0	0
Thiols	c	0	0	0	+	0	0
	1y	0	0	0	+	0	0
Peroxidase	c	0	0	0	0	0	+
	1y	0	0	0	0	0	+
Explained variance		33 %	11 %	12 %	6 %	7 %	5 %

Evaluations of responses within the present study area were performed on the resulting component scores. Since differences between the two years existed, but relations among the trees were consistent in both years (Spearman's correlation coeficients between the two years from 0.7 to 0.9, $P<0.01$ for all single variables), results on the component scores of two years were compiled to a combined data set after a z-transformation of the data for each year.

Differences between sites were calculated by a two-way analysis of variance (ANOVA) with the independent factors altitude (below or above 1200 m.a.s.l.) and "site quality" according to criteria given above (+ or -). Post-hoc-comparisons were completed using the least squared difference method.

Results and Discussion

Statistically significant altitude effects were observed on the scores of components 3 (chlorophyll a/b), 4 (antioxidants), and 5 (chlorophylls/carotenoids) (Table 2). This coincides with previous results on single variables describing altitude dependencies for ascorbate, glutathione, and chloroplast pigment composition (WILDI & LÜTZ 1996, TAUSZ & al. 1994). Spruce needles from higher altitudes generally exhibited higher concentrations of antioxidants and decreased chlorophyll contents, indicating plant responses to "high altitude stress". At higher altitude trees have to cope with combination of high irradiation, low temperatures, and high atmospheric ozone concentrations.

Table 2. Two-way analysis of variance (ANOVA) with the independent factors altitude (below or above 1200 m.a.s.l.) and site type (+ or -). Decision rule for effects: *p<0.05, **p<0.01, ***p<0.005, ns= non-significant.

	Component number					
Effects	1	2	3	4	5	6
Altitude	ns	ns	*	*	*	ns
site type	ns	*	ns	***	ns	ns

The effect of the site type (+ or -) was significant on the scores of component 2 (redox state of carotenoids). Shifts in redox state of carotenoids (α/β-carotene and xantophylls/carotenoids ratios) were repeatedly ascribed as a plant response to an oxidative stress situation (TAUSZ & al. 1994).

The strongest effect was that of the site type on the antioxidant concentrations of component 4 (Table 2, Fig. 1).

These results indicate that in this case study site dependent factors (water regime) dominate over the altitude factor with respect to antioxidative responses in the needles.

An independent support for the hypothesis that site dependent water supply was related to stress responses in these trees was provided by measurements of osmotic needle potentials. They showed a significantly negative correlation to the needle ascorbate concentrations (Fig. 2).

More negative osmotic potenials indicate physiological adaptation to lower water availability. Deficient water supply is an important stress factor inducing plant responses at the physiological level in form of increased formation of phytotoxic oxygen species.

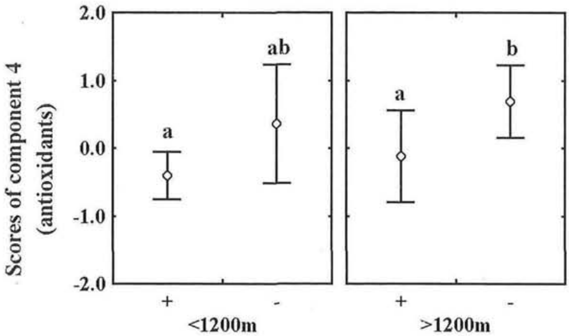

Fig. 1. Scores of component 4 (antioxidants) at two different levels of site quality and altitude. Medians and median deviations of 10 to 16 trees per data point.. Different letters indicate significant differences between sites at the $p<0.05$.

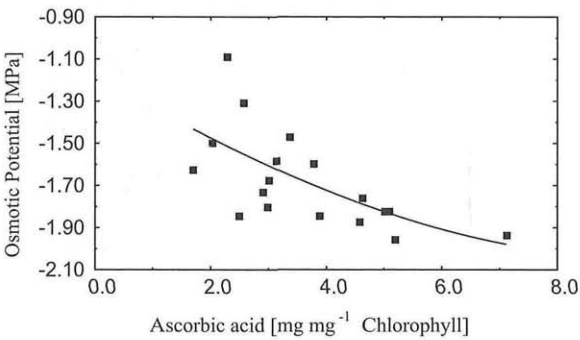

Fig. 2. Osmotic needle potentials and contents of ascorbate in one year old spruce needles. Measurements of osmotic potentials conducted on the 1994 samples only. Spearman's $\rho=-0.63$, $p=0.004$.

Increasing ascorbate concentrations were regarded as responses toward increased oxidative stress to trees (SMIRNOFF 1993).

The present work emphasizes the importance of small scale edaphic factors for the initiation of antioxidative. If such physiological patterns are used for risk assessments of forest systems, these results have to be considered.

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