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ELEMENT CONTENTS AND STRESS-PHYSIOLOGICAL CHARACTERIZATION OF *PINUS CANARIENSIS* NEEDLES IN MEDITERRANEAN TYPE FIELD STANDS IN TENERIFE

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ABSTRACT

Contents of Ca, Mg, K, Na, S, Cl, P, N, pigments, ascorbic acid, glutathione, and tocopherol together with chlorophyll fluorescence were measured in needles of *Pinus canariensis* trees growing at four field stands at 4 different altitudes at the south-eastern slope of the Teide in Tenerife. S contents reflected impacts of SO_2 at lower altitudes. Via Na and Cl contents the influence from the sea was assessed up to 1000 m. Above 1000 m we found higher levels of ascorbic acid, less chlorophyll, higher carotenoid/chlorophyll-ratios, and lower α/β-carotene ratios. This pattern suits in a picture of elevated oxidative stress these trees are responding to. Decreases in Fv/Fm-ratios of chlorophyll fluorescence below 0.80 reflecting damages in the photosynthetic apparatus were only found at one site at 1500 m, where altitude stress was probably amplified by drought stress.

INTRODUCTION

Conifers have been used extensively for bioindication purposes in temperate forests. The analysis of accumulating elements in spruce needles is an important tool for environmental monitoring and threshold values for sulfur, fluoride, and chloride are legally approved in several countries [1]. The nutritional state of forest trees has been evaluated by the analysis of nutrient contents in the needles in numerous studies [2, 3]. In the last years the concepts of stress physiology have been widely accepted and the measurements of stress related variables were included in field studies [4, 5]. Depressions of chlorophyll contents and modifications of pigment compositions were used as physiological bioindicators to assess damage at an early stage [2, 3]. Components of the antioxidative system were found to be useful for the indication of non-accumulative environmental stresses, such as photo-oxidative air pollutants, drought or radiation [2, 5]. Under the influence of these stresses elevated contents of ascorbic acid [5, 6, 7, 8], glutathione [6, 7], or tocopherol [7, 8] could be found. The use of chlorophyll fluorescence measurements has been introduced as a valuable tool in forest decline research under field conditions [9]. However, most of these studies were carried out in temperate forests and it is of great importance if these methods are adequate for mediterranean type forests, especially for the main forest trees. On Canary Islands, the endemic *Pinus canariensis* Chr. Sm. ex DC is an important forest species and intensively used for recultivations.

The aim of this study is to assess the nutritional and stress physiological status of *P. canariensis* trees and the impact of pollutants on these forests by means of nutrient, pigment, and antioxidant analysis combined with chlorophyll fluorescence measurements at selected field stands in Tenerife.

MATERIALS AND METHODS

Plant Material: Investigations were conducted on needles of *P. canariensis*. Four mediterranean type sites at the south-eastern slope of Tenerife island were sampled: site 1, at 550 m a. s. l. near Candelaria town, and sites 2, 3, and 4 in 850, 1500, and 1950 m, respectively, above Arico village. Southward, light-exposed branches were cut in 3 to 4 m height from five dominant old trees per site, and kept watered in the laboratory overnight. Needles were cut under constant light and temperature conditions the next day, immediately frozen in liquid nitrogen and lyophilized for biochemistry, and oven dried at 105 °C for element analysis. Material was ground in a dismembrator and lyophilized powder was stored humidity-proof in the freezer until analysing.

Analyses: S and Cl were measured by ion-exchange HPLC after Schöninger-combustion, N was measured by standard Kjeldahl procedure, P was measured by a colorimetric method, Mg, Na, K, and Ca were determined by atomic absorption spectrophotometry (Shimadzu); pigments, ascorbic acid, and glutathione were measured as cited in [10], α-tocopherol as in [7].

Chlorophyll fluorescence was measured by a portable device (PEA, Hansatech, UK) at needles of harvested branches after keeping them overnight.

Statistics: Statistical evaluation was done by Statistica (StatSoft, USA) software package. Differences between sites were calculated by Kruskal-Wallis-test followed by cross-comparisons according to Schaich and Hamerle [11].

RESULTS AND DISCUSSION

The results of element analyses of the needles are shown in Fig. 1A and 1B. *Pinus*-needles at all four sites contained similar amounts of Mg and K. The needles sampled at site 3 showed significantly lower Ca contents than those at sites 1 and 2. Na and Cl contents were significantly higher at site 1 and 2. Strong correlations between both Na and Cl concentrations (Pearson's $r=0.93$ for $n=20$ trees, $P<0.001$) show that these data reflect the impact of seawater transported via air to the lower elevated sites. P contents were significantly lower at site 4 compared to sites 1 and 2. N contents in the needles were different among sites. It is difficult to evaluate these data because optimum ranges of these elements are still unknown for *P. canariensis*. An investigation on *P. halepensis* revealed comparable contents of P and N and somewhat higher contents of Ca were determined [12]. These authors found pronounced annual variations in contents of N, P, and Mg with minima in late summer and maxima in early spring, regardless of the ozone treatments they performed in their study.

P. canariensis needles at the lower elevated sites 1 and 2 contained much S compared to needle contents of other conifers in field studies, but S values decreased with increasing altitude. Although sulfate uptake through the roots is not known since soil analyses were not performed, it is usually strictly controlled by feedback mechanism. Because big variations in needle S-contents are often caused by entrance of sulfurous gases through the stomata, this variable can be used for bioindication of SO_2 impact [1]. Although S and Cl data are significantly correlated (Pearson's r=0.658, P=0.002) it is unlikely that sulfur was deposed as sulfate deriving from seawater, because Cl/S ratio of seawater is usually over 10 [13]. Even the difference of S-contents between sites 1 and 3 is much bigger. Data strongly point to considerable amounts of SO_2 deposited predominately in lower elevations in this region.

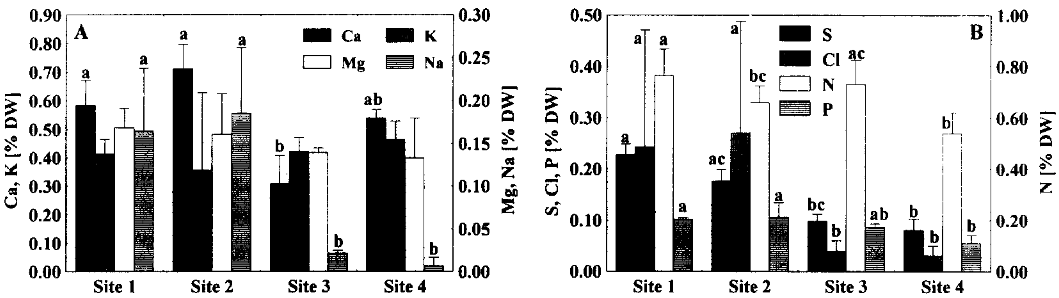

Fig. 1. Element contents of 1 year old *P. canariensis* needles (DW = needle dry weight) at 4 field stands in Tenerife. Columns are medians, error bars are 80%iles. Different letters indicate significant differences between sites for the respective element (P<0.05).

Biochemical data show decreases of chlorophyll contents at site 3 and 4 (Fig 2A). Studies on spruce trees in the Alps revealed decreasing pigment contents at high elevated sites due to increasing levels of oxidative stress [4, 6]. Together with these depressions of chlorophyll needles at sites 3 and 4 exhibited a greater portion of carotenoids and lower α-carotene/β-carotene-ratios. Increases in carotenoid/chlorophyll ratios may reflect an increased need for photoprotection of chlorophylls by carotenoids. This ratio can be used as an early indicator for needle chlorosis [14]. Depressions in α/β-carotene ratios may reflect oxidation processes within the thylakoid membranes which may occur under oxidative stress conditions and were found repeatedly in damaged conifer needles [2, 15]. The epoxidation state of the xanthophyll cycle in the morning was not significantly different among the sites (Fig. 2A, V/VAZ). A higher epoxidation state at this time, which could provide enhanced photoprotection at the cost of photosynthetic efficiency, was found as a consequence of prolonged stress impact [16]. Ascorbic acid contents were significantly higher at sites 3 and 4 than at site 2 (Fig. 2B), which may reflect an adaptation to an increased need for antioxidative protection in high altitudes [4, 6]. However, the levels of the antioxidants glutathione and α-tocopherol did not vary much among the sites (Fig. 2B), although some studies on spruce found responses of these variables to high altitude stresses [6]. Because glutathione is not only an important antioxidant but also the major transport form of reduced sulfur, its concentrations might be also raised by excessive sulfur supply via SO_2 [10]. α-Tocopherol provides antioxidative power directly in the membranes, predominately in chloroplast membranes. Its content might be elevated after massive pollutant attack caused membrane damages, which was obviously not the case in this study.

Fluorescence measured in the morning exhibited decreased Fv/Fm ratios at needles from site 3 (Fig. 2B), which reflects decreased quantum yield at photosystem II, probably as a consequence of stress influence. At sites 1, 2, and 4 Fv/Fm values were near the theoretical optimum of 0.833 (Fig 2B).

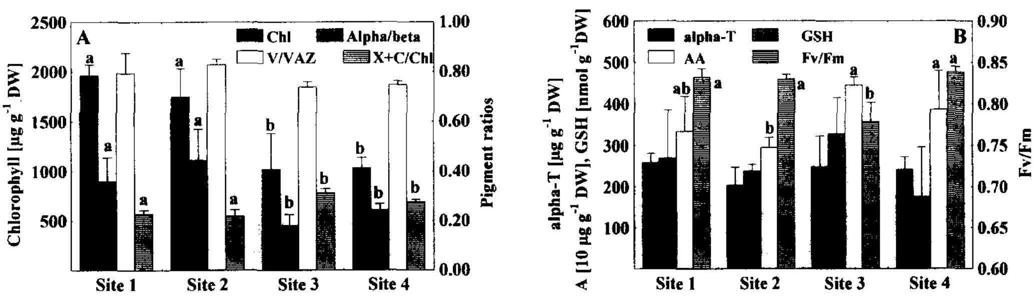

Fig. 2. (A) Total chlorophyll contents (Chl), α-carotene/β-carotene-ratios (Alpha/beta), violaxanthin/xanthophyll-cycle pool ratios (V/VAZ), carotenoids/chlorophylls ratios (X+C/Chl) and (B) α-tocopherol (alpha-T), total ascorbic acid (AA, axis values must be multiplied by 10), total glutathione (GSH), chlorophyll fluorescence (Fv/Fm) of 1 year old *P. canariensis* needles at 4 field stands in Tenerife. DW = needle dry weight. Columns are medians, error bars are 80%iles. Different letters indicate significant differences between sites for the respective variable ($P<0.05$).

Summing up we can say that sites 1 and 2 are clearly influenced by NaCl deriving from seawater. Furthermore, *Pinus* needles at these sites showed dramatically elevated S contents which are probably of anthropogenic origin. Nevertheless, *P. canariensis* seems to cope well with the environmental situations at these sites, because physiological measurements (pigment contents, fluorescence data) did not reveal impairments of metabolism. Reports on responses of antioxidants to SO_2 are contradictory in literature, and we did not find severe alterations in the present study. The situation at sites 3 and 4 was different: As data for Na and Cl contents prove, the influence of seawater was negligible at these sites. The same is true for the probable impact of antropogenic S sources, because S contents of the needles are lower at these sites. On the other hand, chlorophyll contents are lower and ascorbic acid contents are higher than at sites 1 and 2. Together with observed alterations in pigment compositions, (lower α/β-carotene and higher carotenoid/chlorophyll-ratios) these results fit in a model of increasing oxidative stress levels with increasing altitude. Increased ozone levels contribute to environmental stress at high altitudes. In Tenerife, summer 3 month's averages increase steadily from about 25 ppb at sea level to about 55 ppb at 2000 m (E. Cuevas, personal communication). But, as fluorescence data show, even at nearly 2000 m (site 4) *P. canariensis* trees are able to avoid damages in the photosynthetic apparatus. Such damages occurred only at site 3 (at 1500 m). Neither altitude stress, which would be most severe at site 4, alone, nor the impact of sulfur, which occurred at sites 1 and 2, impaired physiological vitality of the investigated trees. Additional factors must be taken into account explaining the situation at site 3. In a mediterranean type ecosystem with pronounced summer droughts, drought stress certainly is an important factor: Smirnoff [17] reported the development of oxidative stress within cells of drought stressed plants. Although *P. canariensis* is certainly well adapted against water loss, prolonged stomatal closure will lead to photostress situation, because CO_2 is not available. Signs of the most extreme drought situation site 3 was exposed to were found by the development of xeromorphoses in the needles (data in preparation), which occur after prolonged drought. However, further evaluation of these data will be necessary.

This study gives insight in the occurances of environmental stresses and the protection mechanisms of *P. canariensis* in Tenerife and helps to explain why these trees are able to grow under dramatically different ecological conditions. The evaluation of the stress status of trees at different field stands should be of interest for the use of *P. canariensis* trees for afforestations at the Canary Islands.

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REFERENCES

1. Austrian Federal Law Gazette, 2. Verordnung gegen forstschädliche Luftverunreinigungen, Bundesgesetzblatt 199, Vienna (1984).
2. M. Tausz, G. Zellnig, E. Bermadinger-Stabentheiner, D. Grill, K. Katzensteiner and G. Glatzel, Physiological, structural, and nutritional parameters of Norway spruce needles from different forest stands in Austria. Can. J. For. Res. **26**, 1769-1780 (1996).
3. R. Oren, K. S. Werk, N. Buchmann and R. Zimmermann, Chlorophyll-nutrient relationships identify nutritionally caused decline in *Picea abies* stands. Can. J. For. Res. **23**, 1187-1195 (1993).
4. A. Polle and H. Rennenberg, Field studies on Norway spruce trees at high altitudes: II. Defence systems against oxidative stress in needles, New Phytol. **121**, 635-642 (1992).
5. M. Tausz, M. Müller, E. Stabentheiner and D. Grill, Stress-physiological investigations and chromosomal analysis on Norway spruce (*Picea abies* (L.) Karst.) - a field study, Phyton (Austria) **34**, 291-308 (1994).
6. M. Tausz, D. Grill and E. Bermadinger-Stabentheiner, Physiological responses of spruce trees to air pollutants - a tool for bioindication. *Proceedings of the 16th International Meeting for Specialists in Air Pollution Effects on Forest Ecosystems. 'Air Pollution & Multiple Stresses.' September 7-9, 1994. Fredericton, New Brunswick, Canada.* Canadian Forest Service - Atlantic Centre (ISBN 0-662-24845-7). pp. 167-173, (1996)
7. B. Wildi and C. Lütz, Antioxidant composition of selected high alpine plant species from different altitudes, Plant Cell Environ. **19**, 138-146 (1996).
8. U. Schmieden and A. Wild, Changes in levels of α-tocopherol and ascorbate in spruce needles at three low mountain sites exposed to Mg^{2+}-deficiency and ozone, Z. Naturforsch. **49c**, 171-180 (1994).
9. T. Saarinen, Chlorophyll fluorescence, and nitrogen and pigment content of Scots pine (*Pinus sylvestris*) needles in polluted urban habitats, Ann. Bot. Fennici **30**, 1-7 (1993).
10. M. Tausz, L. J. De Kok, I. Stulen and D. Grill, Physiological responses of Norway spruce trees to elevated CO_2 and SO_2, J. Plant Physiol. **148**, 362-367 (1996).
11. J. Bortz, G. A. Lienert and K. Boenke, Verteilungsfreie Methoden in der Biostatistik, Springer Verlag, Berlin (1990).
12. S. Elvira, R. Alonso, R. Inclan, V. Bermejo, F. J. Castillo and B. S. Gimeno, Ozone effects on Aleppo pine seedlings (*Pinus halepensis* Mill.) grown in open top chambers, Water Air Soil Poll. **85**, 1387-1392 (1995).
13. W. Larcher, Ökophysiologie der Pflanzen, 5th Edition. p. 14, Ulmer, Stuttgart (1994).
14. H. K. Lichtenthaler, The plant prenyllipids, including carotenoids, chlorophylls, and prenylquinones. In Lipid metabolism in plants (Edited by T. S. Moore). pp. 427-470, CRC Press, Boca Raton, Ann Arbor, London, Tokyo (1993).
15. D. Siefermann-Harms, Light and temperature control of season-dependent changes in the α- and β-carotene content of spruce needles, J. Plant Physiol. **143**, 488-494 (1994).
16. W. W. Adams III and B. Demmig-Adams, Carotenoid composition and down regulation of photosystem II in three conifer species during the winter, Physiol. Plant. **92**, 451-458 (1994).
17. N. Smirnoff, The role of active oxygen in response of plants to water deficit and desiccation, New Phytol. **125**, 27-58 (1993).