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Environmental Stresses and Antioxidative Responses of *Pinus canariensis* at Different Field Stands in Tenerife

By

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Summary

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In the present paper, we investigate antioxidants, chloroplast pigments, ultrastructure, and chlorophyll fluorescence in needles of *Pinus canariensis* Chr. Sm. ex DC. collected at four natural stands covering an altitude range from 200 to 2000 m a. s. l. in the South-East of Tenerife. The contents of chloroplast pigments were lower at the most elevated sites showing chlorophyll concentrations of about 1 mg g^{-1} needle dry weight (DW) versus 2 mg g^{-1} DW at the lowest sites. Mean antioxidant concentrations in one year old needles were 260 to 200 µg g^{-1} DW for α-tocopherol, 3 to 4.5 mg g^{-1} DW for ascorbic acid and 340 to 180 nmol g^{-1} DW for glutathione. A strict altitude dependence of these parameters was not found. Chlorophyll fluorescence measurements revealed Fv/Fm above 0.75 for all sites indicating healthy photosynthetic apparatus, but revealed initial states of oxidative stress at one particular site. Depressions in Fv/Fm went together with decreased α-carotene/β-carotene ratios and a decrease in the ratio of violaxanthin to total xanthophyll cycle carotenoids. Ultrastructural investigations of the chloroplasts indicated no severe damage to the thylakoid systems.

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Introduction

Investigations of stress-physiology and ultrastructure of conifer trees have been the focus of very much interest in temperate forest ecosystems in central Europe. They have revealed modifications of antioxidative defense systems (e. g. GRILL & al. 1989), chloroplast biochemistry and structure (e.g. ZELLNIG & al. 1989), and chlorophyll fluorescence parameters (e. g. SAARINEN & LISKI 1993, TAUSZ & al. 1996) due to oxidative stress developing in response to complex environmental impacts.

The question arose whether conifers in different types of ecosystems show comparable reactions to stress patterns. *Pinus canariensis* is an endemic plant of the Canary Archipelago whose natural distribution area is restricted to the highest islands. In Tenerife it grows spontaneously from near sea level up to about 2.200 m. In the present paper, we investigate antioxidants, chloroplast pigments, ultrastructure, and chlorophyll fluorescence in needles of this conifer growing at different altitudinal levels, since this type of studies have not been realized in this tree species until the present time.

Materials and Methods

Sampling sites: The study was conducted in Tenerife (Canary Islands) 28°35'15"- 27°59'59" Northern latitude and 16°5'27"- 16°55'4" Western longitude. Four sites (I, II, III and IV) of natural pine forest on the Southern slope were established from the lower distribution limit to the upper border line at 550, 850, 1500 and 1950 m altitude respectivelly.

Preparation of needle material: Branches of 40 to 50 year old trees of *P.canariensis* cut in the field during summer, were kept well watered, 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, ascorbic acid and glutathione were measured as cited in TAUSZ & al. 1996, α-tocopherol as in WILDI & LÜTZ 1996.

Chlorophyll fluorescence measurements: The minimal (Fo), maximal (Fm) and variable (Fv) chlorophyll fluorescence were monitored with a Plant Efficiency Analyser, PEA (Hansatech, Norfolk, UK) portable fluorimeter, in the same material used for biochemical analysis, after having been in the dark overnight and water saturated.

Ultrastructure: One year old needle samples were fixed in 2.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 M phosphate buffer at pH 7.2 and 0.1 M saccharose, and postfixed in a 1:1 mixture of 2 % osmium tetroxide in 0.2 M phosphate buffer (pH 7.3) and 4 % potassium cromate. Subsequently they were dehydrated in graded series of ethanol and propylene oxide and embedded in Epon. Staining was done with lead and uranium salt.

Statistics: Statistical evaluations were completed using Statistica (StatSoft, USA, 1994) software package. Differences between sites were calculated by non-parametric Kruskal-Wallis analysis of variance due to small sample sizes (5 trees per site). After Kruskal-Wallis-test proved significance ($P<0.05$), cross-comparisons of sites were completed according to Conover's test (BORTZ & al. 1990). Figures show medians and 20-80%ile range. Different letters in the figures mark significant ($P<0.05$) differences. Correlations between fluorescence data and carotenoid composition were calculated by a complex fitting model indicated in the figures with the quality of fit indicated by the correlation coefficient between predicted and observed values as well as by the portion of variance explained by the model.

Results

The contents of chloroplast pigments in *Pinus canariensis* trees growing at four different altitudinal sites were lower at the most elevated sites showing chlorophyll concentrations of about 1 mg g^{-1} needle dry weight (DW) versus 2 mg g^{-1} at the lowest sites. The changes in the chlorophyll a/b ratio were not so clear with increasing altitude showing a decrease in site III (Fig. 1 A). Mean antioxidant concentrations in one year old needles were 260 to 200 µg g^{-1} DW for α-tocopherol, 3 to 4.5 mg g^{-1} DW for ascorbic acid and 340 to 180 nmol g^{-1} DW for glutathione. A strict altitude dependence of these parameters was not found (Fig. 1, B, C and D). The percentage of dehydroascorbate and oxidized glutathione were high but did not show significant differences among the sites.

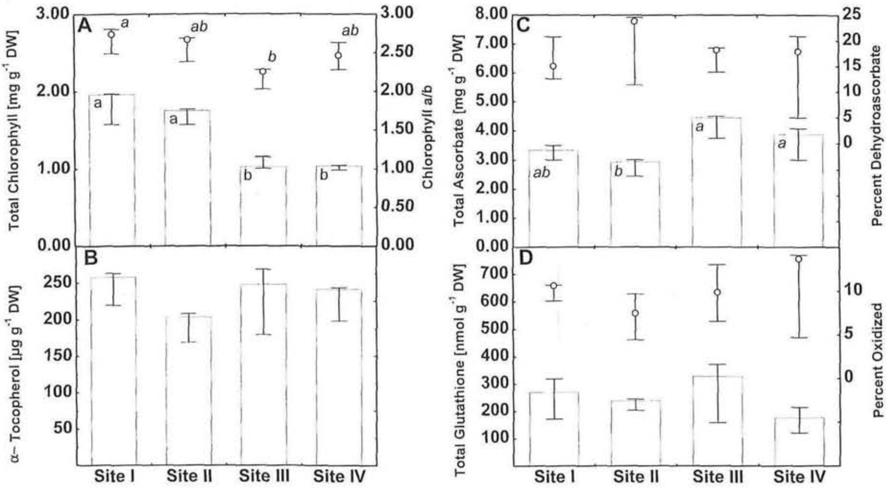

Fig. 1. Chlorophyll and antioxidant contents of one year old *P. canariensis* needles at the four sites. A. Total chlorophylls (chlorophyll a+b, columns) and chlorophyll a/b ratios (O). B. α-Tocopherol (columns). C. Total ascorbic acid (oxidized + reduced, columns) and dehydroasorbic acid as percent of total (O). D. Total glutathione (reduced + oxidized, columns) and oxidized glutathione as percent of total glutathione (O).

Chlorophyll fluorescence measurements revealed F_v/F_m ratios above 0.83 for all sites with the exception of site III which showed values a little lower (0.77). This decrease was due to the lowering of Fm and not to changes in Fo values (Fig. 2). Depressions in F_v/F_m went together with decreased α-carotene/β-carotene ratios and a decrease in the ratio of violaxanthin to total xanthophyll cycle carotenoids (violaxanthin + antheraxanthin + zeaxanthin). (Fig. 3).

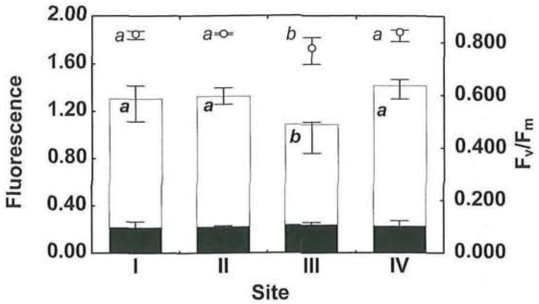

Fig. 2. Chlorophyll fluorescence (in relative units) of one year old *P. canariensis* needles at the four sites. Filled columns = F_0; open columns = F_v; total columns = F_m; O = F_v/F_m.

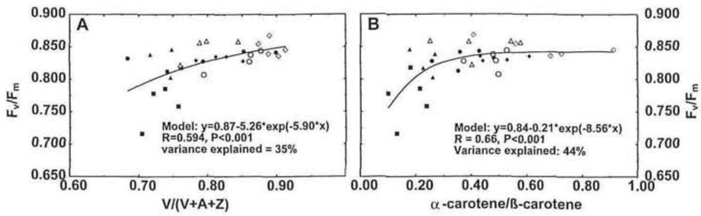

Fig. 3. Correlations between carotenoid composition and chlorophyll fluorescence. ●, O site I, ◆, ◇ site II, ■ site III, ▲, △ site IV. Open symbols = current year's needles, filled symbols = one year old needles. A. State of the xanthophyll cycle versus F_v/F_m. V = Violaxanthin, A = Antheraxanthin, Z = Zeaxanthin. B. α-Carotene/β-Carotene ratios versus F_v/F_m.

On the ultrastructural level no characteristic differences were visible in the mesophyll chloroplasts of the four sites. In general, the chloroplasts were in a good condition and did not show severe damage in their main structural parameters like thylakoid development, plastoglobuli number, starch content and stroma density (Fig. 4).

Discussion

Studies in temperate and coniferous forest ecosystems have shown an altitude dependent accumulation of antioxidant in plants (WILDI & LÜTZ 1996) and a decrease in pigment content (TRANQUILLINI 1964, TODARIA & al. 1980) . The altitudinal decrease of chlorophyll content in needles of *Pinus canariensis* trees is in agreement with the general literature although not with a previous paper donewith small canary pine plants growing in pots at 600 and 2100 m altitude (MORALES & al. 1982) in which the opposite trend was shown. This fact together with the lack of correlation with the altitude of the chlorophyll a/b ratio

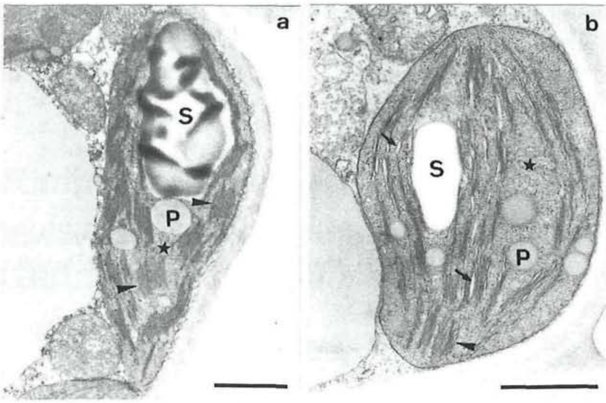

Fig. 4. Electron micrographs of mesophyll chloroplasts of needles from site I (a) and III (b). The chloroplasts show many grana thylakoids (arrowheads), some electron-light plastoglobuli (P), starch grains (S) and a normal appearance of the stroma (*). (b) Sometimes also slight swellings of the thylakoids (arrows) can be noticed. Scale bars = 1 μm

and antioxidant content in the present results, induces us to think that the observed changes in chlorophyll and antioxidant content in this species could be more dependent on environmental factors rather than altitude.

The existence of many different microclimates in the Canary Islands is well known. The influence of the trade winds coming from the North East bringing in moisture from the sea forms a zone of clouds in the middle elevations varying its altitudinal situation along the year and being more variable on the Southern slope of the island, where the four experimental sites are situated. This masks the effect of the altitude being probably the different environmental factors the causes of stress at the sites. We applied the chlorophyll fluorescence technique because it is a good indicator of stress in plants (LICHTENTHALER 1988, BOLHÁR-NORDENKAMPF & al. 1989, LARCHER & al. 1991) The Fv/Fm values obtained at all the sites were in the range considered as normal (0.75 to 0.85) in leaves (BOLHÁR-NORDENKAMPF & LECHNER 1990), this together with the ultrastuctural findings indicate that pine trees were not suffering any great stress. The small decrease of the Fv/Fm ratio at site III was not accompanied by any alteration in the photosynthetic apparatus, the Fo value remained constant and only the Fm and therefore the Fv decreased slightly. Lower values of the Fv/Fm ratio in the morning brought about decreases in the epoxidation state of xanthophyll cycle and decreases in a α-carotene/β-carotene ratios, this may reflect initial states of oxidative stress due to environmental conditions at the site (probably drought stress). The retention of zeaxanthin and antheraxanthin during the night was found by ADAMS & DEMMIG-ADAMS 1994 in response to low temperature in other coniferous species.

From the results of this work in which the sampling was done in summer, under very extreme hot and dry conditions, we conclude that *P. canariensis* is well adapted to living in its natural distribution area not presenting severe stress symptoms in any of the studied sites. To know the cause of the variations in the pigments and antioxidants contents, more studies have to be done including environmental factors at the sites.

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References

ADAMS W. W. III & DEMMIG-ADAMS B. 1994. Carotenoid composition and down regulation of photosystem II in three conifer species during the winter. - Physiol. Plant. 92: 451-458.

BOLHÁR-NORDENKAMPF H.R.& LECHNER E.G. 1990. The determination of the "photosynthetic capacity" using chlorophyll fluorescence to measure stress levels in leaves. - In: Trends in photosynthesis research, advanced course. (Lectures). Palma de Mallorca (Spain), p. 71-75.

— , LONG S. P., BAKER N. R., ÖQUIST G., SCHREIBER U.& LECHNER E. G. 1989. Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. - Functional Ecology 3: 497-514.

BORTZ J., LIENERT G.A. & BOENKE K. 1990. Verteilungsfreie Methoden in der Biostatistik. - Springer Verlag, Berlin, Heidelberg, New York.

GRILL D., GUTTENBERGER H., ZELLNIG G. & BERMADINGER E. 1989. Reactions of plant cells on air pollution. - Phyton (Austria) 29(2): 277-290.

LARCHER W., WAGNER J., NEUNER G., MÉNDEZ M., JIMÉNEZ M. S. & MORALES D. 1991. Thermal limits of photosynthetic function and viability of leaves of *Persea indica* and *Persea americana*. - Acta Oecologica 12: 529-541.

LICHTENTHALER H.K. 1988. In vivo chlorophyll fluorescence as a tool for stress detection in plants. - In: LICHTENTHALER H. K. (ed.), Applications of chlorophyll fluorescence, pp. 129-142 - Kluwer Academic Publishers.

MORALES D., JIMÉNEZ M.S., IRIARTE J. & GIL F. 1982. Altitudinal effects on chlorophyll and carotenoid concentrations in gymnosperms leaves. - Photosynthetica 16(3): 362-372.

SAARINEN T. & LISKI J. 1993. The effect of industrial air pollution on chlorophyll fluorescence and pigment contents of scots pine (*Pinus sylvestris*) needles. - Eur. J. For. Path. 23: 353-361.

TAUSZ M., DE KOK L. J., STULEN I. & GRILL D. 1996. Physiological responses of Norway spruce trees to elevated CO_2 and SO_2. - J. Plant Physiol. 148: 262-367.

TRANQUILLINI W. 1964. The physiology of plants at high altitudes. - Ann. Rev. Plant Physiol. 15: 345-362.

TODARIA N. P., THAPLIYAL A. P. & PUROHIT A. N. 1980. Altitudinal effects on chlorophyll and cartenoid contents in plants. - Photosynthetica 14: 236-238.

WILDI B. & LÜTZ C. 1996. Antioxidant composition of selected high alpine plant species from different altitudes. - Plant Cell Environ. 19: 138-146.

ZELLNIG G., GAILHOFER M., PFEIFHOFER H. W. & GRILL D. 1989. Ultrastructure and pigment composition of chloroplasts of differently damaged green and yellowed needles of *Picea abies*. - Phyton (Austria) 29 (2): 213-225.